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**COMPARISON OF NEW ZEALAND (SOUTH ISLAND) AND  
AUSTRALIAN (TASMANIAN) WALNUT CULTIVARS:  
AN ORGANOLEPTIC AND BIOCHEMICAL STUDY.**

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A thesis  
submitted in partial fulfilment  
of the requirements for the Degree of  
Master of Science

at

Lincoln University

by

Leo P. Vanhanen

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Lincoln University

New Zealand

2010

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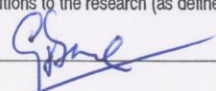
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**Abstract of a thesis submitted in partial fulfilment of the requirements for the  
Degree of Master of Science**

**COMPARISON OF NEW ZEALAND (SOUTH ISLAND) AND AUSTRALIAN  
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AN ORGANOLEPTIC AND BIOCHEMICAL STUDY.**

By Leo P. Vanhanen

Seven different cultivars of walnuts (*Juglans regia* L.) grown in Australia (Tasmania) were compared with 6 cultivars grown in New Zealand (Canterbury). A preliminary assessment of the physical attributes and taste of all the nuts was carried out by members of the New Zealand Walnut Industry Group. The overall assessment scores of the Tasmanian walnuts was 73 (out of 100) compared to 65 for walnuts grown in Canterbury. While most of the attributes between the two groups of nuts were similar, the overall crackout score of the Tasmanian nuts received a perfect score of 20 while the Canterbury walnuts scored a mean of 14 for all of the six cultivars.

The two groups of walnuts were then subjected to organoleptic assessment using 59 untrained tasters. The tasters recorded their impressions of the taste and after taste of all the nuts using a five point hedonic scale. The overall taste and after taste scores for the Tasmanian and Canterbury walnuts were very similar but individual cultivars Earnsclough, Vina and Fernette achieved higher scores for taste when compared with all of the other cultivars evaluated while Fernette grown in Tasmania was given the highest overall score for the attribute after taste. Overall the cultivar Fernette grown in Tasmania had the best mean scores for both taste (3.5) and after taste (3.5) which were significantly greater than all of the other cultivars.

All the walnuts were subjected to general proximate analysis, individual fatty acid analysis, tocopherol and phytosterol profile, and total and free phenolic analysis.

There were no conclusive differences between Australian and New Zealand grown walnuts when comparing the proximate analysis, fatty acid profile, tocopherol profile, phytosterol profile or phenolic analysis.

This is the first time the total and free phenolic content of New Zealand and Australian walnut cultivars has been reported. The free phenolic levels ranged from 10.9 to 16.6 mg gallic acid equivalents (GAE)/g dry weight (DW) of walnut kernel, for cultivars Kirwee and Howard respectively. The total phenolic level ranged from 14.0 to 24.7 mg GAE/g DW of walnut kernel, for cultivars Serr (New Zealand) and Vina (New Zealand), respectively.

There were no unusual observations or statistical differences when comparing the organoleptic and biochemical parameters between the different walnut cultivars.

A correlation between the organoleptic and biochemical results was performed which showed no statistically significant correlations between any of the parameters determined.

**Keywords:** Walnut, *Juglans*, sensory evaluation, taste, consumer panel, biochemistry New Zealand, South Island, Australia, Tasmania.

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*“Avoid fruits and nuts. You are what you eat.”*

*Jim Davis, Actor, 1909 -1981*

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And last but not least I wish to say thanks to my immediate family in Christchurch, Cheann, Oscar, Isabella and Joan.

I do believe both scientifically and non-scientifically that we are what we eat !

and Walnuts are great to eat !.

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## **Chapter 1**

### **Introduction**

In the late 1970's the first walnut cultivar comparison was started in New Zealand and in 1985 and 1987 replicated trials at Lincoln University compared horticultural performance of walnuts sourced from Europe, The United States of America and New Zealand (McNeil and Savage, 2001). From these trials better performing cultivars were selected for commercial production.

Since then no variety comparisons of local or imported cultivars have been performed.

The walnut industry in New Zealand has arrived at the cusp of requiring more investment and development. The New Zealand Walnut Improvement Group (NZWIG) has identified the need to import new walnut cultivars into New Zealand to diversify the New Zealand selection, with the objectives of improving plant quality and shelled walnut quality.

There are numerous new large walnut orchards being planted or to be planted at present and one of the keystone questions is what cultivar to plant? This can be answered on two levels, from a horticultural perspective or from a food science perspective, as the ultimate end product is a high value food.

To perform proper growth trials would require planting a large range of cultivars, monitoring during growth and taking the first harvest in 6 to 8 years. This becomes a long term research project and a costly exercise.

In the absence of growth trials being set up it is proposed to assess potential new cultivars organoleptically and biochemically.

The food science approach may not replace the ultimate horticultural approach, but it will help to make a more educated, scientific and "based on fact" decision to which cultivars would be favourable to be introduced into New Zealand.

This approach also has the advantage it will take substantially less time and cost less.

Australia has a more advanced walnut industry, compared to New Zealand, and grows both similar and different cultivars with varying commercial success. Tasmania is known for its quality of the walnuts it grows. One of the reasons why Tasmania produces quality walnuts is its temperate climate, which is often compared to the climate in the South Island of New Zealand.

Walnut growers and other horticulturists have commented on the similar weather and growing conditions between the two regions (Tasmania and the South Island). This point was made clearly in a report to NZWIG after a visit to a number of walnut orchards in the Swansea area, near Launceston in Tasmania (Clive Marsh, 2009, pers. comm.).

However other potential differences may occur due to differing horticultural practices, such as irrigation, fertilisation and pruning regimes.

A comparison of the climatic data recorded at the Lincoln University weather station (S43°38'41", E172°27'22") and Swansea Post Office, Tasmania (S42°7'25", E148°4'30", Australian Bureau of Meteorology) can be seen in Table 1. The Swansea Post Office weather station was chosen as this is the closest site to Tasmania's major walnut producer, Webster's Walnuts, main orchard of 550 hectares.

Table 1.1 Comparison of climate statistics, from Lincoln University, Canterbury, New Zealand and Swansea Post Office, Tasmania, Australia.

	Lincoln		Swansea	
		Years <sup>1</sup>		Years
Mean rainfall (mm/year)	643.7	39	593.3	125
Mean solar exposure (MJ/m <sup>2</sup> )	13.4	39	14.7	19
Mean maximum temperature (°C)	16.6	39	17.9	52
Mean minimum temperature (°C)	6.3	39	7.8	52

<sup>1</sup> Years of data collection.

It is proposed to sample 7 different cultivars of walnuts harvested at maturity from commercial orchards in Tasmania, Australia and 6 different cultivars from the South Island of New Zealand, were chosen to be assessed.

These will then be analysed for biochemical quality parameters: total fat, total protein, total fibre, fatty acid profile, tocopherol profile, phytosterol profile and physiological parameters such as shape characteristics, shell seal and percent crackout.

The walnuts will also be subject to taste and appearance evaluation. The data sets will then be statistically analysed to establish any correlations and inter-relationships

To conclude, this research was initiated and was funded by the Walnut Industry Group (WIG) to assist them with their objectives of promotion of quality standards, education, collaboration and increasing knowledge of walnut taste properties, composition and nutritional quality, for all walnut growers.

## **1.2 Objective and hypotheses**

### **1.2.1 Objective 1. Organoleptic comparison.**

The comparison between different cultivars and the same cultivars grown in both locations, was carried out to investigate whether there are organoleptic differences between walnut cultivars grown in Tasmania compared to those in the South Island of New Zealand ?.

#### **Hypothesis 1.**

The organoleptic evaluation of walnuts has been used previously, for example, to evaluate the quality of  $\gamma$ -irradiated walnuts (Mexis and Kontominas, 2009) and it has also been used to evaluate the taste of walnuts in packaging and storage condition experiments (Mexis *et al.*, 2009; Jensen *et al.*, 2003).

Sinesio and Moneta (1996) established groupings of walnuts by geographical regions, using PCA analysis of the sensory evaluation descriptors they used to evaluate the walnuts. The importance of the walnut kernel flavour/taste and selection of the corresponding cultivar(s), is highlighted in all of the preceding reports.

It is hypothesised that at least 1 or more new walnut cultivar(s) will score highly in the organoleptic testing and therefore be identified as a potential importation candidate(s).

It is also hypothesised that since the growing conditions are similar, there will only be minor taste differences between the same and different cultivars grown in the 2 locations.

### **1.2.1 Objective 2. Biochemical comparison.**

Determine any biochemical differences between cultivars grown in Tasmanian compared to South Island. Are there differences between cultivars ?.

## **Hypothesis 2.**

The biochemical properties of walnuts are generally well known and recorded (USDA, ARS Nutrient database, 2009). The Biochemical properties of most of the New Zealand grown walnut cultivars has been determined previously by Zwarts *et al.* (1999) and Savage (2001). These sorts of investigations have also been carried out by other researchers, on walnut cultivars grown in their home countries, for example Ruggeri *et al.* (1998), determined the chemical composition of walnuts grown in Italy. Sze-Tao and Sathe (2000) reported the proximate composition of walnuts grown California, USA.

There are few, if any inter-country comparisons of walnut biochemical compositions. Therefore it is proposed to compare the biochemical profile of the walnuts grown in Tasmania to those grown in the South Island, this will include cultivars that are the same and different. Since the meso-climate of the two regions is very similar it is hypothesised the biochemical profile will be very similar.

It is also proposed to compare and correlate the biochemical profile of the walnuts analysed to the organoleptic results.

The correlation of visual appearance to taste of the walnut has been done by Colarič *et al.* (2006), but they found no correlations. They found the assessors had quite different perceptions in tasting the kernels.

It is hypothesised that one or more significantly higher scoring organoleptically walnuts will have a significantly different biochemical profile and this will be identified.

### **1.2.3 Objective 3. The scientific evaluation of in-shell and shelled walnuts.**

What are the quality parameters that define an in-shell and shelled walnuts, as defined by different countries and international organisations. Compare and make suggestions with respect to the international standards.

## **Hypothesis 3.**

In modern food production the quality parameters of the food are important, as they need to be matched to the type of post-harvest processing used and the final end product use.

All foods available in New Zealand and Australia are regulated by the Australian and New Zealand Food Standards Code (ANZ FSC , 2009). A standard for walnuts can be found in Standard 2.3.1 Fruits and Vegetables , which includes nuts (Appendix A.1).

There are other standards for walnuts, the United Nations Economic and Social Council (UN ESC) have recently published a standard for in-shell walnuts (UN ESC, 2008). The United States Department of Agriculture (USDA) has standards for shelled and in-shell walnuts however these are quite old 1968 and 1976 (USDA, 1968, 1976), copies can be found in Appendix A.2, 3 and 4 respectively.

Balta *et al.* (2007) defined the physical characteristics of walnuts grown in the Bitlis province of Turkey. In this study 17 different cultivars had seven different shell characteristics determined, such as shell thickness and seven different shelled nut characteristics, such as nut colour. There are few published details documenting accurate data such as this.

In the late 1970's the then Walnut Action Group (WAG) originally imported walnut cultivars into New Zealand. The WAG created an assessment guide to evaluate the quality of the inshell and shelled walnuts from the first harvests from these trees. This same walnut assessment guide will be used to evaluate the overall quality of the walnuts proposed to be imported into New Zealand from Tasmania, in 2009.

The guide covers seven points, which are primarily of importance to processing the nut, but do also indicate the overall quality of the nut.

It is hypothesised that the comparison of the WAG walnut assessment guide will not correlate well with the results of the organoleptic evaluation (Objective 1) and the biochemical profile (Objective 2). As the WAG walnut assessment guide is only performed by a small number of people, the scoring method used is not as statistically robust and there are no scientific analyses carried out as part of the assessment.

## Chapter 2

### Literature Review

#### 2.1 Organoleptic study of walnuts

The organoleptic study of walnuts could include not only the taste but also the visual appearance and physical size of the walnut.

The organoleptic evaluation (tasting) of walnuts has been used previously, for example, to evaluate the quality of  $\gamma$ -irradiated walnuts (Mexis and Kontominas, 2009) and it has also been used to evaluate the taste of walnuts in packaging and storage condition experiments (Mexis *et al.*, 2009; Jensen *et al.*, 2003).

A full descriptive analysis was carried out by eight highly trained panellists to determine differences between Persian (*Juglans regia* L.) and black (*Juglans nigra* L.) walnuts grown in United States of America (Warmund *et al.*, 2009). The panellists agreed upon one aroma, 11 flavour and 10 textural descriptive terms and definitions to evaluate the walnuts on.

Bujdosó *et al.* (2006) organoleptically tested a range of Hungarian cultivars and potential candidates for registration on the Hungarian National List, to find out whether any given variety is more appealing in the shell or kernel. They used basic taste, skin colour, skin surface and kernel colour, to evaluate the Hungarian walnut cultivars. An expert panel was used to judge the walnuts.

Colarič *et al.* (2006) used six descriptors for external appearance and six descriptors for taste to evaluate differences between ten different walnut cultivars grown in Slovenia, with a panel of 40 non-expert tasters. Some of the cultivars were the same as those grown in Tasmania, Australia and used in our trial.

Sinesio and Moneta (1997) did an in-depth descriptive analysis of six different cultivars, eighteen samples in total, from five different countries (France, Greece, Italy, Portugal, Spain). The panel consisted of seven highly trained assessors, whom agreed on 15 sensory variables to describe the visual appearance and flavour of walnut kernels.

The importance of the walnut kernel flavour/taste and selection of the corresponding cultivar(s), is highlighted in all of the preceding reports.

Sinesio and Moneta (1997) established groupings of walnuts by geographical regions, using Principal Component Analysis (PCA) analysis. The sensory evaluation descriptors Sinesio

and Moneta (1997) used were able to distinguish and group walnuts from different countries, there were also different countries within the same groups. Therefore walnuts grown in different countries are likely to taste different, however some countries did show similarities in taste.

## **2.2 Biochemical study of walnuts**

The biochemical and chemical composition of walnuts, in general has been well studied, however the composition of properly named different cultivars of walnuts has not occurred until relatively recently.

Ruggeri *et al.* (1998) is the earliest journal reference concerning the chemical composition of named walnut cultivars. In this study the proximate analysis, soluble sugar content and amino acid content of the walnut cultivars Sorrento, Franquette, Hartley and Trento were reported. The context of this research was the comparison of different walnut cultivars to other tree nut crops grown in Italy. This theme of research is common when walnuts are studied.

The general proximate analysis of 12 different cultivars of walnuts grown in New Zealand is reported by Savage (2001).

The crude protein ranged between 13.6 to 18.1, the total lipid 62.6 to 70.3, ash 1.9 to 2.4, starch 1.5 to 2.8, dietary fiber 3.1 to 5.2, ADF 2.2 to 3.8 and NDF 3.3 to 4.4 g /100g DM walnut.

This range of proximate measurements included five cultivars grown in New Zealand of European and United States origin. All these values are comparable to values quoted by Ruggeri *et al.* (1998). No similar studies specifically on Australian grown cultivars have been performed.

### **Fatty acid profile**

The walnut kernel has been reported to contained between 52 to 70% total lipid (Prasad, 1994). This is dependent on the type of cultivar, growing location and other growing variables , such as irrigation.

There are normally five fatty acids found in walnuts at a level above 1% relative total composition, for example palmitic, stearic, oleic, linoleic and linolenic.



Li *et al.* (2007) identified 30 different fatty acids that were below 1% relative composition, in Combe Persian walnuts. The fatty acid profile of New Zealand walnuts has been done by Savage *et al.* (2001), who identified the common five and two more, below 1% relative composition, these are D-vaccenic and gadoleic.

A study by Zwarts *et al.* (1999) on the fatty acid content of walnuts grown in New Zealand, concludes that New Zealand grown walnuts had a distinct fatty acid profile. The New Zealand grown walnuts had a fatty acid profile that was more variable, compared to the fatty acid profiles of walnuts from European and USA grown nuts.

There have been no specific studies on the fatty acid profile of Australian grown walnuts.

All of the bioactive compounds to be found and identified in walnuts has not yet been achieved, however there are several groups of compounds responsible for bioactivity that have, these are tocopherols, phytosterols and phenols.

### **Tocopherol**

Alpha,  $\beta$ ,  $\gamma$  and  $\delta$  tocopherols have been identified in walnuts from around the world (Amaral *et al.*, 2005; Crews *et al.*, 2005; Li *et al.*, 2007, Savage, 2000). No tocotrienols have been detected in walnuts from New Zealand (Savage, 2000) and Crews *et al.* (2005) did not detect any tocotrienols in authentic walnut oils from China, France, Hungary, India, Italy, Spain and USA.

Amaral *et al.* (2005) studied nine different cultivars from two different geographical locations in Portugal and found  $\gamma$ -tocotrienol in all samples. The amounts ranged from 2.25 to 5.14 mg/kg compared to total tocopherols in walnuts ranging from 194 to 632 mg/kg.

The small amount of tocotrienols present compared to the total tocopherols and the fact Amaral *et al.* (2005) detected tocotrienols across a range of different cultivars, suggests other researchers have perhaps not used an analytical method sensitive enough.

Amaral (2005) compared the tocopherol content of walnuts from 2 geographical locations in the same country (Portugal) and found differences in the tocopherol content, however these were not significant. Significant differences were observed when cultivars were grouped by the year of production, indicating differences were due not only due to genetic factors but influenced by environmental factors as well.

## Phytosterol

The analysis and identification of phytosterols in walnuts is not commonly done. Several researchers have analysed walnut oils for phytosterols (Martinez *et al*, 2006; Schwartz *et al*, 2008; Vanhanen *et al*, 2005). Since sterols are only found in the lipid fraction it is valid to use such data for comparison.

The total phytosterol content of walnuts has been reported to range from 1,060 mg/kg – 1,770 mg/kg by Philips *et al*. (2005), 2,030 to 2,855 mg/kg by Savage *et al*. (2001) from 12 different New Zealand cultivars, 1,129.5 mg/kg by Maguire *et al*. (2004) and 2,340 mg/kg by Schwartz (2008). This indicates there is a lot a variability in phytosterol amounts in walnuts grown from around the world. It is not known if this is due to differences in geographical locations, climate or genotype differences

In all the literature the most predominate phytosterol is  $\beta$ -sitosterol, but similarly the percentage contribution has a large range, this being 65%, 78% 66% and 62% of the total sterols.

Again no conclusions can be made about the variation in the  $\beta$ -sitosterol constant as there have been no studies performed specifically addressing this.

## Phenols

Phenols in walnuts could be classified as a plant phytochemical (Chen and Blumberg, 2008), as are phytosterols, carotenoids and other groups of compounds that may affect a person's health either positively or negatively.

Phenols in plant derived foods are a very diverse group of compounds. To date it is estimated that there are more than 6,000 different types of phenols (Taiz and Zeiger, 2002). Phenolics can be found in all parts of a food, for example, the shell, leaf and kernel of a walnut. With varying amounts and types of different phenols from different parts of the walnut.

Based on their fundamental chemical structure phenols can be divided into two major groups called non-flavonoids and flavonoids. Within these two major groups there are many different sub-groups.

Very little , if not any, research has been done to identify and characterising walnut kernel phenols.

Liu *et al.* (2008) has identified three diarylheptanoids and an  $\alpha$ -tetralone in green walnut husks of an unspecified cultivar. Olvereira *et al.* (2008) has reported the total phenolic content of aqueous extracts of green walnut husks from five cultivars found in Portugal.

Pereira *et al.* (2007) has reported the phenolic profile of walnut leaves, identifying 10 aqueously extracted phenolic compounds from six different walnut cultivars grown in Portugal. It is important to note that walnut green husks and leaves are not normally eaten.

Researchers have reported the total phenolic content of walnut kernels, however most of the studies are not specific to walnut cultivars, and are done as a consequence of surveying a range of nuts or similar food types (Arcan and Yemenicioğlu, 2009; Chen and Blumberg, 2008; Gunduc and El, 2003; Kornsteiner *et al.* 2006). Arcan and Yemenicioğlu (2009) reported a range of 175 to 414 mg gallic acid/100g dry weight walnuts. Gunduc and El (2003) reported 7,051.74 mg catechin /kg walnut. Chen and Blumberg (2008) and Kornsteiner *et al.* (2006) both report similar amounts, 16.3 mg gallic acid equivalents/g and 1,625 gallic acid equivalents /100 g fresh weight walnuts, respectively. In both Chen and Blumberg (2008) and Kornsteiner *et al.* (2006) walnuts had the highest total phenolic content compared to all the other nuts in their studies (almonds, brazil nuts, cashew, hazelnuts, macadamias, peanuts, pecans, pine nuts and pistachios).

Pereira *et al.* (2008) analysed six different cultivars of walnuts grown in Portugal, values ranged from 60.83 to 95.06 mg gallic acid equivalents/g walnut extract.

There is no published data on the phenolic profile or total phenolic content of different walnut cultivar kernels grown in New Zealand or Australia.

## **Chapter 3**

### **Materials and Methods**

#### **3.1 Walnut selection**

A total of 13 different cultivars of walnuts (*Juglans regia* L.) in the shell were chosen for this study. Seven cultivars from trees growing in Tasmania, supplied by Webster's Walnuts, Tasmania, Australia: Chandler, Fernette, Fernor, Howard, Lara, Serr and Vina. They were imported to the Lincoln University, Ministry of Agriculture and Forestry (New Zealand), PC1 accredited facilities. All organoleptic and biochemical assessments on the imported walnuts were carried out in Lincoln University PC1 accredited faculties. Any unused walnuts, shells, kernels or any waste was autoclaved prior to disposal.

Six cultivars of walnuts, currently grown in New Zealand were supplied by Cracker of a Nut, West Melton, New Zealand: Rex, Meyric, Serr, Vina, Kirwee and Earnsclough.

#### **3.2 Organoleptic Analysis**

##### **3.2.1 Initial assessment**

A group of six New Zealand Walnut Industry Group committee members carried out an initial physical and taste evaluation of the Australian (Tasmania) walnuts. As a result of this initial assessment of the walnuts imported from Australia the parameters for the later organoleptic tests were decided upon for all of the walnuts.

The initial assessments carried out were:

1. Shell Attractiveness.

This is an overall visual assessment on the appearance of the in-shell walnut. Taking into account the smoothness, colour, shape and size. This was scored out of 20 points.

2. Shell seal and strength.

Twenty walnuts were dropped from shoulder height onto a concrete paver and then manually squeezed to test whether the walnut seal opened. One point is scored for every walnut that is firmly intact.

3. Crackout %.

Crackout percentages were calculated by weighing 20 randomly selected walnuts in the shell, the kernels were then manually separated from the shell and weighed. The ratio of walnut kernel to whole walnut is then expressed as a percentage. Any result over 50% or over gets 20 points. For results below 50%, 2 points are taken off, a starting value 20 points, for every 1% down on 50%.

4. Ease of extraction.

Does the walnut kernel come easily out of the shell? Scored out of 10 points.

5. Kernel shape.

Does the kernel come easily into halves? Is there uniformity and consistency in the half shape? Scored out of 10 points.

6. Kernel flavour.

The overall flavour of the walnut kernel was scored out of 10 points. Ten being the most pleasant tasting.

7. Kernel colour.

The overall colour of the walnut kernel was scored out of 10 points. Ten points having a honey blond colour.

The scores are then added up to 100 points and each cultivar is given an overall mark out of 100. A copy of the evaluation sheet used can be seen in Appendix A.5. The walnuts were freshly hand cracked and labelled using a random 3 digit number as seen in Figures 1 and 2 .

### **3.2.2 Organoleptic assessment**

All 13 different walnuts were freshly shelled and subjected to a panel tasting using a 5 point hedonic scale, to measure taste (initial or first taste) and after taste (taste in the mouth after a few seconds). The words chosen to anchor the scales were, flavoursome - very pleasant, flavoursome, mild taste, tasteless - bland, awful, for both taste and after taste. With 5 being flavoursome and 1 being awful. A copy of the evaluation sheet used can be seen in Appendix A.6.

Walnuts were labelled using a 3-digit random number and presented in a random order to panellists in plain white polystyrene cups.

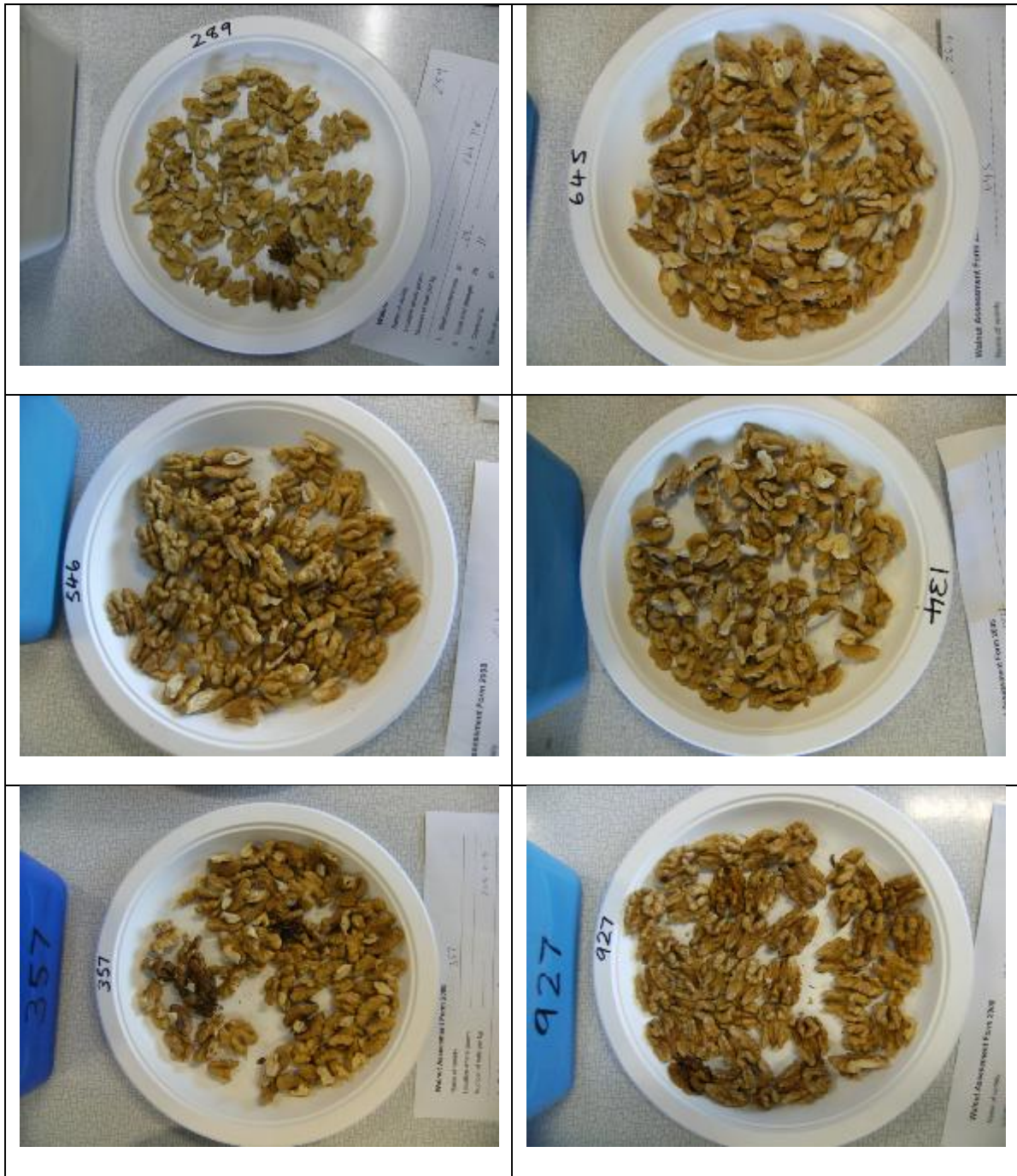


Figure 3.1 Coded New Zealand walnuts.



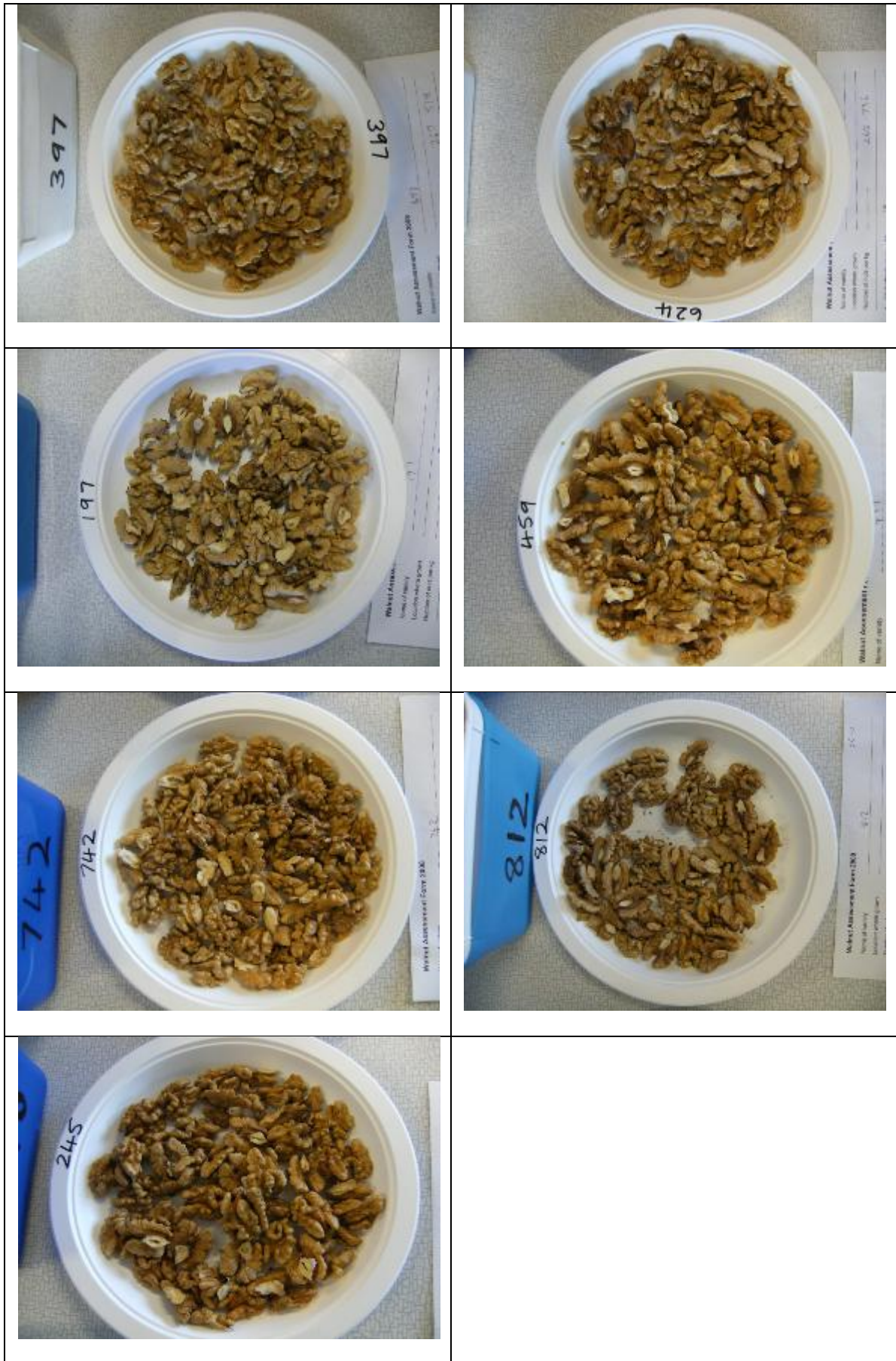


Figure 3.2 Coded Australian walnuts.

Panellists were asked to work in pairs, with one member of the pair being blindfolded, the other presenting the walnuts and recording the response. No communication was allowed between the pairs and a micro-break with the consumption of water was enforced between each walnut (Figure 3.3).

A total of 59 panellists performed the evaluation. Panellists were sourced from the Canterbury Branch of the New Zealand Walnut Association and from staff and students of Lincoln University, New Zealand.



Figure 3.3 Organoleptic assessment of walnuts.

### 3.2.3 Statistics

All results were collated with Microsoft ® Excel 2003 and statistical analysis was performed using Minitab 15 ® ver. 15.1.0.0. 2006.

A one-way ANOVA was carried out to determine the specific statistical differences on the mean results of the data sets.

Descriptive statistical tests were also performed on the data to indicate dispersion, skewness and help indicate any trends.



### 3.3 Biochemical analysis

All chemicals and solvents used in the following analyses were of analytical grade and purchased from VWR International Ltd, Leicestershire, U.K. unless otherwise stated.

#### 3.3.1 Lipid extraction.

Lipid samples were extracted from the freshly cracked in-shell walnut kernels, using a modified method of Savage *et al.* (1997). In brief, approximately 5g of kernel was extracted for 1 hour, in 30 mL of hexane/isopropanol (3:2 v/v) at room temperature, in steel tubes containing four stainless steel ball bearings. The homogenate was then centrifuged and the supernatant washed with 20 mL of 6.7% w/v aqueous sodium sulphate.

The supernatant was then transferred into 100 mL round-bottom flasks and evaporated to dryness using a Büchi Rotovapor-R (Postfach, Switzerland) set at 35°C, samples were stored under nitrogen gas (oxygen free) at -20°C prior to analysis.

#### 3.3.2 Proximate analysis.

The following proximate analyses were performed on freshly cracked walnut kernels. Moisture was determined gravimetrically, by drying in a oven at 100°C for 48 hours. Ash content was determined gravimetrically by incinerating in a muffle furnace for 16 hours at 550 °C.

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined gravimetrically using the method used by Van Soest (1991). Total lipid content was determined gravimetrically, using an automated soxhlet extraction (Tecator Soxtec 1043) and petroleum ether as the solvent. Total crude protein was determined using an automated kjeldahl process (Tecator Kjeltac 1035).

#### 3.3.3 Fatty acid analysis.

Lipid samples from the extracted walnut kernels were methylated according to the method described in Savage *et al.* (1997). In brief, 10 mg of extracted lipid was accurately weighed and dissolved into 500 µL of hexane. Two mL of 0.01M NaOH in dry methanol (SeccoSolv,

Merck, Darmstadt, Germany) was added to the sample and incubated for 10 minutes at 60 °C. To esterify, 3 mL of 20% boron trifluoride methanol complex (Merck, Darmstadt, Germany) was added and incubated for a further 10 minutes at 60 °C. Two mL of 20% aqueous NaCl was then added and 1 mL of hexane. The sample was then centrifuged and the hexane layer removed and stored under N<sub>2</sub> (oxygen free) and -20 °C prior to immediate analysis.

Methylation was confirmed by spotting 10 µL onto a silica gel 60, glass thin-layer chromatography plate (Merck, Darmstadt, Germany). The plate was run with hexane:diethyl ether:acetic acid (85:15:1, v/v/v) as a mobile phase and developed by spraying with molybdate phosphoric acid in 10% ethanol:ether (1:1, v/v) and heating for 15 minutes at 120 °C.

Analysis of the fatty acid methyl esters (FAME) was performed on a Shimadzu gas chromatograph GC-2010 with a AOC-20i auto-injector and flame ionization detector. A Hewlett Packard INNO-Wax capillary column (Agilent Technologies, Santa Clara, CA, United States), 30 M long with an internal diameter of 0.25 mm and film thickness of 0.25 µm was used for analysis.

One µL of methylated sample was injected at a split ratio of 30:1 into the injection port, held at 230 °C. The carrier gas used was Helium (zero grade) held at a constant flow rate of 0.80 mL/min. Initially the column was held at 160 °C for 5 minutes, then the temperature was increased at a rate of 1.6 °C per minute until reaching a maximum temperature of 240 °C.

Peak identification was performed by comparison of peak retention times to commercially prepared FAME standards 15A and 68A (Nu-Chek Prep, Inc. Elysian, MN, United States). Raw data capture and peak integration was performed using Shimadzu Lab Solutions ver 2.3.

### 3.3.4 Tocopherol analysis.

Tocopherols were analysed by HPLC according to the method described by Dutta *et al.* (1994). Lipid extracts were diluted (1:100) with n-heptane (LiChrosolv, BDH, UK). The diluted lipid sample was then injected into a Waters 7725 Rheodyne injector with a 10 µL sample loop and 510 HPLC pump (Waters, Milford, USA). A Varian LC 9070 fluorescence detector (Varian, Walnut Creek, USA) was used for the detection of tocopherols at wavelengths of 294 nm and 320 nm for excitation and emission, respectively. A Lichrospher 100 NH<sub>2</sub>, (250 x 4 mm, particle size 5 µm, Merck, Darmstadt, Germany) coupled to a LiChroCART 4-4 (Merck, Darmstadt, Germany) guard column was used to analyse the

samples. An isocratic elution was carried out using a mixture of heptane:tert-butylmethylether:tetrahydrofuran:methanol (LiChrosolv, Merck, Darmstadt, Germany) (79:20:0.98:0.02, v/v/v/v) at a flowrate of 1.2 mL/minute. Identification of peaks was performed by the comparison of retention times to a standard reference mix of  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  tocopherols (Supleco, Bellefonte, PA, USA) and  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  tocotrienols, which were kindly supplied by Professor P.D. Dutta of SLU, Sweden. Integration of peaks was accomplished by a HP 3396A integrator (Hewlett-Packard, Avondale, USA). External standard curves were used for the quantification of tocopherols and tocotrienols by dilution to four concentrations, 1, 5, 10 and 50 ng/10  $\mu$ L.

### 3.3.5 Phytosterol analysis.

Phytosterol analysis of extracted lipids was performed according to the method described in Damirchi *et al.*, (2005). In brief, 20 mg of lipid was saponified with 1 mL of 2M potassium hydroxide at 60°C for 45 minutes. To this 1 mL of water, 2 mL of hexane and 200  $\mu$ L of absolute ethanol was added, and mixed thoroughly. The hexane layer is removed and evaporated to dryness under oxygen free nitrogen gas. Trimethylsilyl (TMS) ether derivatives were then prepared to enable the GLC analysis of the phytosterols. To each sample 100  $\mu$ L of Tri-Sil reagent (Pierce Chemical Co. Rockford, USA) was added and incubated for 45 minutes at 60°C. The remaining solvent is evaporated under oxygen free nitrogen gas, then 500  $\mu$ L of hexane is added in preparation for gas chromatographic analysis.

Gas chromatographic analysis was carried out on the silylated sample using a fused silica capillary column DB-5Ms 30 m x 0.25 mm, 0.50  $\mu$ m (J&W Scientific, Folsom, USA). The column was connected to a Chrompack CP 9001 gas chromatograph (Chrompack, Middelburg, The Netherlands) with a flame ionization detector. The oven was initially set at 60°C for 1 minute then increased at a rate of 40°C/minute, to a maximum temperature of 310°C for 27 minutes. Quantification was performed by using 5 $\alpha$ -cholestane as an internal standard and results calculated relative to  $\alpha$ -cholestane. Peak areas were calculated using Maestro version 2.4 (Chrompack, Middelburg, The Netherlands).

The internal standard, 5 $\alpha$ -cholestane, was prepared by accurately weighing 5 mg of 5 $\alpha$ -cholestane (Sigma, St Louis, USA) into a 25 mL volumetric flask and making up to the volume with hexane. Seventy five  $\mu$ L of this was added to each sample, approximately 15  $\mu$ g of internal standard per sample.

### 3.3.6 Free and total phenols.

A modified method of Imeh and Khokhar (2002) was used to extract the free and total (conjugated) fractions of the walnut kernel.

Freshly cracked in-shell walnut kernels were frozen (-20°C) then coarsely ground in a coffee grinder (Sunbeam, Australia). The ground samples were then freeze dried. After freeze drying they were ground further in a coffee grinder (Sunbeam, Australia) to a fine powder and 1 g was accurately weighed into a 50 mL screwcap test tube.

To this 20 mL of 50% v/v methanol:deionised water is added, this was then vortexed for 10 seconds and extracted with gentle agitation in the dark at 60°C for 120 minutes. This is the free phenolic extract.

For total (conjugated) phenolic extract, the procedure above was replicated, using 1.2 M HCl in 50% v/v methanol: deionised water instead of 50% v/v methanol: deionised water.

Both the free and total phenolic extracts were then quantitatively transferred into a 50 mL volumetric flask and made up to volume with the appropriate solvent.

Extracts were then centrifuged at 2,885 RCF and analysed immediately. The phenolic content of both extracts was measured using Folin-Ciocalteu reagent (Sigma, St Louis, USA) according to a method adapted from Singleton *et al.* (1974).

To 0.5 mL of sample extract, 2.5 mL of 0.2 N Folin-Ciocalteu reagent and 2.0 mL of 7.5% w/v sodium carbonate solution (aqueous) were added to a 20 mL screwcap test tube. This was then vortexed for 5 seconds and incubated for 5 minutes at 50°C. The test-tubes were then immediately cooled to room temperature before reading the absorbance at 760 nm on a Unicam Helios-beta UV-VIS spectrophotometer (Unicam, Cambridge, UK).

A standard curve was prepared in duplicate by making a standard solution of 1 mg/mL gallic acid (Sigma, St Louis, USA) in 50% v/v methanol:deionised water and diluting to final concentrations of 0, 40, 60, 80, 120, 160 and 200 µg gallic acid/mL. Samples were appropriately diluted to fit within the standard curve. The final results are expressed as mg gallic acid equivalents per 100 g dry matter (mg GAE/100 g DM).

### **3.3.7 Statistical analysis.**

All Statistical analysis and calculations were performed using Minitab version 15.1 and Microsoft® Office Excel 2003. Means were calculated and a one-way ANOVA used to determine any statistical differences between the mean values.

## **Chapter 4**

### **Results**

#### **4.1 Organoleptic Assessment**

##### **4.1.1 Initial assessment**

A summary of the initial assessment results can be seen in Table 4.1. The total score was calculated from a sum of all the attributes evaluated and is out of a total score of 100. Using this evaluation system the highest scoring walnuts were Meyric, New Zealand (81.0) and Howard, Australia (80.8). The lowest scoring walnut was Serr, New Zealand (52.3).

The overall mean value for the Australian nuts was 73.0, this was higher than the overall mean value for all the New Zealand nuts (64.8).

##### **4.1.2 Organoleptic assessment**

A total of 59 responses were used for statistical analysis of the organoleptic assessments. There were three response forms that were incorrectly filled out, which were not used at all and discarded.

Of the 59 responses used for analysis, there were no smokers. 39% of the panellists were male and 61% female. The age groups were divided into: <19, 19-25, 26-30, 31-50 and >50 years, with 0%, 31%, 15%, 8% and 46% of the panellists in each category respectively.

20% of the panellists had their coffee strong, 39% medium, 17% weak and 24% did not drink coffee at all.

12% of panellists drank their tea strong, 52% medium, 24% weak and 12% did not drink tea.

12% of panellists ate a lot of spicy food, 35% had a medium amount, 51% a small amount and 2% ate none.

Table 4.1 Initial evaluation results comparing mean assessment scores of New Zealand (Canterbury) to Australian (Tasmania) walnut cultivars.

	Cultivar	Shell attractiveness (20) <sup>†</sup>	Shell seal and strength* (20)	Crackout score <sup>#</sup> (20)	Ease of extraction (10)	Kernel			Total score (100)
						Shape (10)	Flavour (10)	Colour (10)	
New Zealand	Earnsclough	19	11	0	8	3	7.2	10	58.2
	Kirwee	17	16	12	8	5	5.2	10	73.2
	Meyric	16	12	20	9	9	6.0	9	81.0
	Rex	16	19	6	4	1	4.5	10	60.5
	Serr	12	8	16	4	2	3.3	7	52.3
	Vina	10	17	18	6	7	3.8	2	63.8
	Mean	15	13.8	12	6.5	4.5	5.0	8	64.8
Australia	Chandler	20	3	20	10	10	4.3	10	77.3
	Fernette	18	9	20	6	4	5.7	8	70.7
	Fernor	14	10	20	8	6	6.2	7	71.2
	Howard	18	15	20	7	8	6.8	6	80.8
	Lara	17	13	20	4	8	5.7	6	73.7
	Serr	12	8	20	9	9	3.8	4	65.8
	Vina	10	18	20	4	9	5.2	5	71.2
	Mean	7	10.9	20	6.9	7.7	5.4	6.6	73.0

<sup>†</sup> Maximum points for each attribute.

\* Dropped from height, intact shell scores 1 point.

<sup>#</sup> Over 50% crackout, scores 20 points. 2 points taken off for every 1% below 50% crackout.

The mean taste and after taste scores for each walnut cultivar can be seen tabulated in Table 4.2. Overall there was no significant difference between the overall New Zealand mean scores compared to Australian mean scores of 3.2 and 3.1 respectively for taste and mean scores of 2.9 and 2.9 respectively for after taste (Table 4.2). This was done using a one-way ANVOA of the means at a 90% level of confidence.

The highest score for the taste attribute was 3.5 for Fernette, Australia, the lowest 2.8 for Meyric, New Zealand and Serr, Australia.

The highest score for the after taste attribute was 3.1 for Serr, New Zealand and Lara, Australia.

Table 4.2 shows the mean individual cultivar scores for the two attributes tested. Additional to this each individual cultivars response was subjected to one-way ANVOA, testing each individual cultivars mean to each other, to determine any statistical differences (raw data not shown). The level of confidence chosen was 90%, as, when the test was performed at 95 or 99% there were no clear statistical differences. The statistically significant scores between cultivars can be seen tabulated as a matrix in Tables 4.3 and 4.4.

Table 4.2 Comparison of mean scores ( $\pm$ SE) for taste and after taste attributes for different New Zealand (Canterbury) and Australian (Tasmania) walnut cultivars.

	Cultivar	Taste	After Taste
New Zealand	Earnsclough	3.4	2.9
	Kirwee	3.3	2.9
	Meyric	2.8	2.8
	Rex	3.1	2.9
	Serr	3.1	3.1
	Vina	3.3	3.0
	Mean	3.2 $\pm$ 0.05	2.9 $\pm$ 0.06
Australia	Chandler	3.2	2.9
	Fernette	3.5	3.5
	Fernor	2.9	2.8
	Howard	3.2	2.9
	Lara	3.3	3.1
	Serr	2.8	2.4
	Vina	3.1	2.7
	Mean	3.1 $\pm$ 0.05	2.9 $\pm$ 0.06)



Table 4.3 Significant differences of mean taste scores for New Zealand (Canterbury) and Australian (Tasmania) walnut cultivars.

		Australia							New Zealand					
		Vina	Serr	Lara	Howard	Fernor	Fernette	Chandler	Vina	Serr	Rex	Meyric	Kirwee	Earnscleugh
New Zealand	Earnscleugh		*			*						*		
	Kirwee		*			*						*		
	Meyric			*	*		*	*	*					
	Rex						*							
	Serr						*							
	Vina		*											
Australia	Chandler		*											
	Fernette		*			*								
	Fernor			*										
	Howard		*											
	Lara		*											
	Serr	*												
	Vina													

\*Indicates significant difference, Fishers LSD,  $p < 0.10$ .

Table 4.4 Significant differences of mean after taste scores for New Zealand (Canterbury) and Australian (Tasmania) walnut cultivars.

		Australia							New Zealand					
		Vina	Serr	Lara	Howard	Fernor	Fernette	Chandler	Vina	Serr	Rex	Meyric	Kirwee	Earnscleugh
New Zealand	Earnscleugh		*				*							
	Kirwee		*				*							
	Meyric						*							
	Rex		*				*							
	Serr		*				*							
	Vina		*				*							
Australia	Chandler		*				*							
	Fernette	*	*	*	*	*								
	Fernor		*											
	Howard		*											
	Lara		*											
	Serr													
	Vina													

\*Indicates significant difference, Fishers LSD,  $p < 0.10$ .

The taste score significant differences (Table 4.3) show no one cultivar was predominately different to another, although there were some individual differences in scores. Serr, Australia (2.8), has the most significant differences, being similar only to Meyric (2.8), Rex (3.1), Serr (New Zealand, 3.1), Fernor (2.9) and Vina (Australia, 3.1).

For the after taste score significant differences, Table 4.4, Fernette (3.5) was significantly different to all of the other cultivars and Serr (Australia, 2.4) was significantly different to all other cultivars apart from Meyric (2.8) and Vina, (Australia, 2.7).

An alternative method of presenting the organoleptic results is to plot the whole set of data as a boxplot. Boxplots are used to show trends and patterns in the data. A boxplot shows six different statistical attributes in one diagram. These are the median, upper and lower quartiles, minimum and maximum and outliers. Figures 4.1 and 4.2 show the boxplots for the taste and after taste score results respectively.

From Figure 4.1 it can be seen Earnsclough, Vina (New Zealand) and Fernette taste scores are exhibiting a trend of higher scores than all the other cultivars.

All the other cultivars are more evenly distributed apart from Meyric which is trending towards a lower taste score.

Fernette is the only cultivar showing a higher after taste score (Figure 4.2). With all the other cultivars being evenly distributed, apart from Meyric and Serr (Australia) exhibiting a trend towards a lower after taste score.

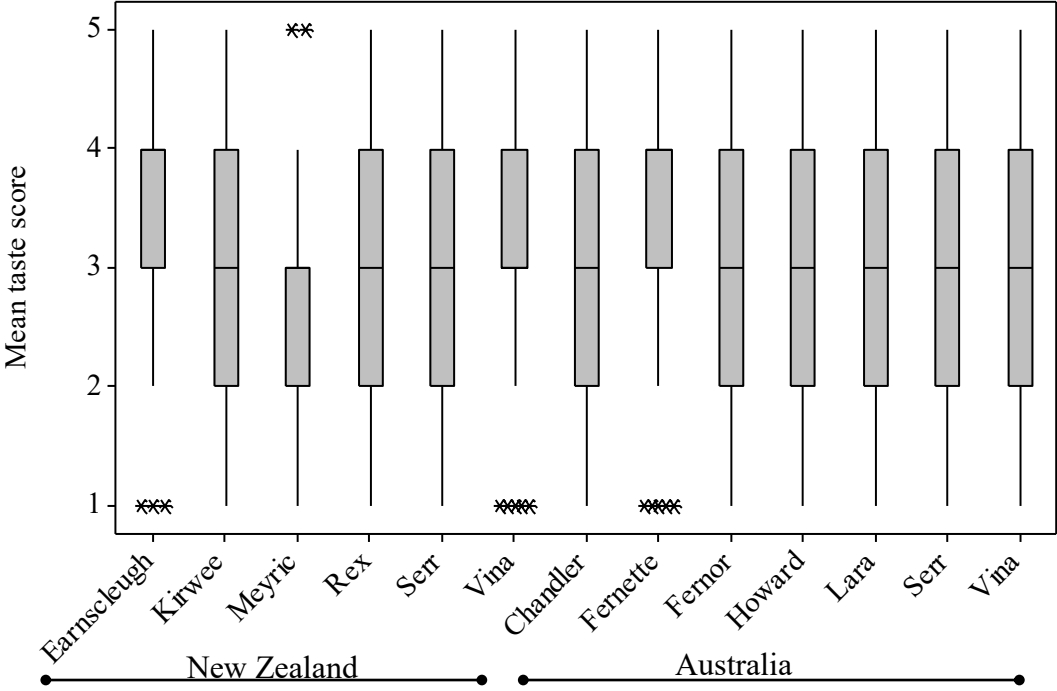


Figure 4.1 Boxplot of mean taste scores for New Zealand (Canterbury) and Australian (Tasmania) walnut cultivars.

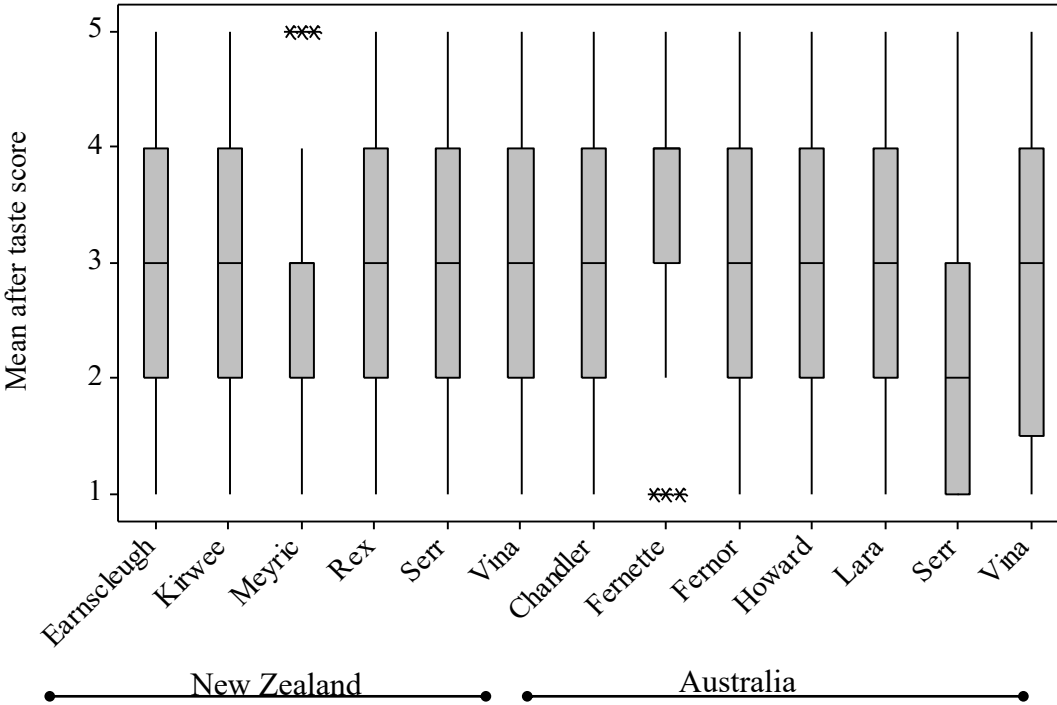


Figure 4.2 Boxplot of mean after taste scores for New Zealand (Canterbury) and Australian (Tasmania) walnut cultivars.

Table 4.5 Mean walnut weights and crackout percentages ( $\pm$ SE) of New Zealand (Canterbury) compared to Australian (Tasmanian) walnuts.

	Cultivar	Nuts <sup>1</sup> per kg	Mean nut weight (g)	Crackout (%)	
				Shell	kernel
New Zealand	Earnsclough	117	8.5	61.0	35.0
	Kirwee	103	9.7	52.8	46.0
	Meyric	106	9.4	49.1	52.6
	Rex	112	8.9	43.1	48.1
	Serr	127	7.9	49.4	48.0
	Vina	122	8.2	46.3	48.8
	Mean	114.5 $\pm$ 3.8	8.7 $\pm$ 0.3	50.3 $\pm$ 2.5	46.4 $\pm$ 1.8
Australia	Chandler	94	10.6	49.5	49.6
	Fernette	88	11.4	47.5	51.1
	Fernor	91	11.0	43.0	51.8
	Howard	90	11.1	44.0	54.0
	Lara	88	11.4	46.3	51.9
	Serr	119	8.4	42.1	52.8
	Vina	87	11.5	43.7	53.4
	Mean	93.8 $\pm$ 4.2	10.7 $\pm$ 0.4	45.1 $\pm$ 1.1	52.1 $\pm$ 0.5

<sup>1</sup> =shell and kernel. All results are mean values of 20 determinations.

A crackout of 20 randomly selected walnuts was performed (Table 4.5) to enable a comparison to data from the initial walnut evaluation (Table 4.1).

The Australian walnuts had larger kernels overall when compared to the New Zealand walnuts, 52.1% and 46.4% respectively and a greater mean nut weight, 10.7 g compared to 8.7 g.

The New Zealand walnut, Earnsclough had the least kernel (35%) and greatest amount of shell (61%).

## 4.2 Biochemical Analysis

The proximate analysis for all the walnut cultivars can be seen in Table 4.6. The overall mean values for dry matter, ash, lipid, crude protein, ADF and NDF of the New Zealand cultivars, compared to the Australian cultivars were all within one standard error. Apart from the NDF mean measurements of  $3.6 \pm 0.3$  and  $3.8 \pm 0.4$  for the New Zealand and Australian cultivars respectively.

Table 4.6 Comparison of proximate analysis (% fresh weight,  $\pm$ SE) of New Zealand (Canterbury) to Australian (Tasmanian) walnuts.

	Cultivar	Dry Matter	Ash	Lipid	Crude Protein	ADF*	NDF†
New Zealand	Earnscleugh	97.5	2.2	65.6	16.7	1.9	3.9
	Kirwee	97.3	2.2	67.5	15.2	1.9	3.2
	Meyric	97.5	1.9	67.9	14.8	2.3	4.0
	Rex	98.2	1.2	66.6	17.4	1.9	3.8
	Serr	98.5	2.1	68.5	13.6	2.2	3.6
	Vina	96.5	2.4	62.6	19.3	1.7	2.9
	Mean	$97.6 \pm 0.5$	$2.0 \pm 0.3$	$66.5 \pm 1.5$	$16.2 \pm 1.5$	$2.0 \pm 0.1$	$3.6 \pm 0.3$
Australia	Chandler	98.5	1.8	65.3	15.3	2.9	4.0
	Fernette	97.8	1.8	67.5	15.4	2.2	4.2
	Fernor	97.7	1.6	67.7	15.1	2.2	4.0
	Howard	97.7	1.8	65.6	17.1	2.4	4.2
	Lara	98.2	2.0	69.7	14.4	2.1	3.0
	Serr	97.5	1.9	69.2	15.3	1.9	2.8
	Vina	97.9	1.7	65.7	16.4	2.1	4.1
	Mean	$97.9 \pm 0.2$	$1.8 \pm 0.1$	$67.2 \pm 1.3$	$15.6 \pm 0.6$	$2.3 \pm 0.2$	$3.8 \pm 0.4$

\* ADF = acid detergent fibre. † NDF = neutral detergent fibre, All results are mean values of duplicate determinations.

Table 4.7 Comparison of mean phenolic (mg gallic acid equivalents/g dry weight walnut kernel,  $\pm$ SE) content of New Zealand (Canterbury) to Australian (Tasmanian) walnuts\*.

	Cultivar	Phenols	
		Free	Total
New Zealand	Earnsleugh	$11.9 \pm 1.2^{c,d,e}$	$15.7 \pm 1.2^{c,d}$
	Kirwee	$10.9 \pm 0.2^e$	$18.6 \pm 1.1^b$
	Meyric	$14.4 \pm 1.2^{a,b}$	$17.9 \pm 0.3^{b,c}$
	Rex	$13.3 \pm 0.4^{b,c}$	$16.0 \pm 0.7^{c,d}$
	Serr	$11.5 \pm 0.5^{c,d,e}$	$14.0 \pm 0.7^d$
	Vina	$13.1 \pm 0.5^{b,c,d}$	$24.7 \pm 0.3^a$
	Mean	$12.5 \pm 0.7$	$17.5 \pm 0.7$
Australia	Chandler	$11.3 \pm 0.6^{c,d,e}$	$15.6 \pm 0.3^d$
	Fernette	$11.1 \pm 0.6^{d,e}$	$14.8 \pm 0.7^d$
	Fernor	$16.0 \pm 0.6^a$	$23.6 \pm 1.1^a$
	Howard	$16.6 \pm 0.5^a$	$19.5 \pm 0.4^b$
	Lara	$13.0 \pm 0.5^{b,c,d,e}$	$14.4 \pm 0.7^d$
	Serr	$11.2 \pm 0.8^{c,d,e}$	$16.1 \pm 0.5^{c,d}$
	Vina	$14.4 \pm 0.9^{a,b}$	$19.5 \pm 0.1^b$
	Mean	$13.2 \pm 0.6$	$17.5 \pm 0.5$

\* All results are mean values of triplicate determinations.

Mean values in the same column that do not share the same letter are significantly different (Fishers Least Significant Difference, 95% individual confidence interval).

The overall free phenolic mean (12.8 mg GAE/g) was lower than the overall total phenolic mean (17.5 mg GAE/g), Table 4.7.

There was no difference between the New Zealand and Australian mean total phenolic content, 17.5 mg GAE/g for both countries.

The mean free phenolic content of the New Zealand walnuts (12.5 mg GAE/g) was slightly lower than the Australian mean of 13.2 mg GAE/g, but within 1 standard error of each other.

Vina (NZ) had the highest total phenolic content (24.7 mg GAE/g) and Serr (NZ) the lowest (14.0 mg GAE/g).

Table 4.8 Comparison of mean tocopherol content (mg/100g oil,  $\pm$ SE) of New Zealand (Canterbury) to Australian (Tasmanian) walnuts\*.

	Cultivar	Tocopherol				Total
		$\alpha$	$\beta$	$\gamma$	$\delta$	
New Zealand	Earnsclough	1.5	0.5	31.4	4.0	37.3
	Kirwee	2.0	0.7	30.0	4.8	37.5
	Meyric	1.6	0.0	30.8	4.3	36.7
	Rex	1.2	0.0	32.5	3.8	37.6
	Serr	1.6	0.0	28.6	4.2	34.4
	Vina	1.8	0.2	30.2	4.2	36.4
Mean		$1.7 \pm 0.1$	$0.5 \pm 0.1$	$31.3 \pm 0.5$	$4.2 \pm 0.1$	$36.6 \pm 0.5$
Australia	Chandler	2.0	0.0	30.6	3.5	36.0
	Fernette	2.1	0.0	28.5	3.7	34.4
	Fernor	1.5	0.0	29.5	4.1	35.1
	Howard	1.7	0.0	35.3	3.2	40.2
	Lara	2.1	0.0	30.5	3.7	36.3
	Serr	1.8	0.3	25.4	2.8	30.3
	Vina	1.8	0.5	28.7	3.5	34.5
Mean		$1.9 \pm 0.1$	$0.4 \pm 0.1$	$29.8 \pm 1.1$	$3.5 \pm 0.2$	$35.3 \pm 1.1$

\* All results are mean values of duplicate determinations.

All four tocopherols were identified in the cultivars, Earnsclough, Kirwee, Vina (NZ), Serr (AUS) and Vina (AUS) as shown in Table 4.8. In the remaining walnut cultivars only alpha, beta and delta tocopherol were identified.

The New Zealand cultivars had a mean total tocopherol content, slightly higher than the Australian cultivars, 36.6 and 35.3 mg/100g oil, respectively.

Howard had the highest amount of total tocopherols 40.2 mg/100g oil and Serr (AUS) the lowest, being 30.3 mg/100g oil.

The ratios of the different forms of tocopherols is similar between each individual cultivars and between countries, apart from the tocopherol forms that are not present. The most predominant tocopherol is gamma-tocopherol ranging from 80.0 to 87.8% of total tocopherol content.

No tocotrienols were identified in any of the samples.



Table 4.9 Comparison of mean fatty acid profile (% ,  $\pm$ SE) of New Zealand (Canterbury) to Australian (Tasmanian) walnuts \*.

	Cultivar	16:0	18:0	18:1(n-9)	18:2(n-6)	18:3(n-3)	SFA	MUFA	PUFA	Ratio $\omega 6/\omega 3$
		Palmitic	Stearic	Oleic	Linoleic	Linolenic				
New Zealand	Earnsclough	7.0	2.3	15.8	60.8	14.0	9.4	15.8	74.8	4.3
	Kirwee	6.3	1.6	17.0	62.8	12.3	7.9	17.0	75.1	5.1
	Meyric	5.8	2.3	20.0	59.5	12.4	8.1	20.0	71.9	4.8
	Rex	5.8	1.6	12.9	64.1	15.6	7.4	12.9	79.7	4.1
	Serr	6.3	2.2	16.6	59.3	15.6	8.5	16.6	74.9	3.8
	Vina	5.9	2.2	18.4	61.0	12.5	8.1	18.4	73.5	4.9
Mean		6.2 $\pm$ 0.2	2.0 $\pm$ 0.1	16.8 $\pm$ 0.9	61.2 $\pm$ 0.8	13.7 $\pm$ 0.6	8.2 $\pm$ 0.3	16.8 $\pm$ 0.9	75.0 $\pm$ 1.1	4.5 $\pm$ 0.2
Australia	Chandler	6.0	1.5	14.9	63.4	14.2	7.5	14.9	77.5	4.5
	Fernette	6.7	2.0	18.2	63.6	12.4	5.8	18.2	76.0	5.1
	Fernor	6.6	1.6	19.9	62.6	9.3	8.3	19.9	71.8	6.7
	Howard	6.2	1.2	13.1	63.1	16.3	7.5	13.1	79.3	3.9
	Lara	5.9	2.0	17.0	61.4	13.7	7.9	17.0	75.0	4.5
	Serr	5.9	2.0	21.5	59.4	11.2	7.9	21.5	70.6	5.3
	Vina	5.4	2.3	21.7	59.4	11.2	7.7	21.7	70.6	5.3
Mean		6.1 $\pm$ 0.2	1.8 $\pm$ 0.2	18.0 $\pm$ 1.2	61.6 $\pm$ 0.6	12.6 $\pm$ 0.9	7.9 $\pm$ 0.2	18.0 $\pm$ 1.2	74.1 $\pm$ 1.3	5.0 $\pm$ 0.3

\*Results are mean values of duplicate determinations. Fatty acids below 1.0% were not identified.

SFA = total saturated fatty acid, MUFA = total mono-unsaturated fatty acid, PUFA = total poly-unsaturated fatty acid.

The most abundant fatty acid from all cultivars was Linoleic acid, with a range of 59.3 to 63.6%, Table 4.9. Compared to literature values there were no unusual observations with respect to the fatty acid profile. Comparing the overall means of the New Zealand and Australian fatty acids, they are almost nearly identical.

Four phytosterols, campesterol, stigmasterol, sitosterol and D<sup>5</sup>-avenasterol were identified in the majority of the walnuts (Table 4.10). Stigmasterol was not detected in Meyric (New Zealand), Lara (Australia) and Vina (Australia).

The total phytosterol content ranged from 135.6 to 199.5 mg/100g oil for Vina (New Zealand and Serr (New Zealand) respectively. Comparing the total phytosterol content of New Zealand to Australia, it would appear the New Zealand walnut has a higher overall mean total phytosterol content, 171.1 compared to 159.9 mg/100g oil.

Table 4.10 Comparison of mean phytosterol content (mg/100g oil,  $\pm$ SE) of New Zealand (Canterbury) to Australian (Tasmanian) walnuts\*.

	Cultivar	Campesterol	Stigmasterol	Sitosterol	D <sup>5</sup> - Avenasterol	Total
New Zealand	Earnsclough	6.2	0.5	135.4	13.2	155.3
	Kirwee	6.4	0.3	122.1	16.8	145.6
	Meyric	6.2	nd	148.9	20.4	175.5
	Rex	9.3	0.4	156.2	28.7	194.6
	Serr	6.5	0.0	158.4	34.5	199.5
	Vina	6.9	0.2	134.7	13.9	155.8
	Mean	6.9 $\pm$ 0.5	0.2 $\pm$ 0.1	142.6 $\pm$ 5.8	21.3 $\pm$ 3.5	171.1 $\pm$ 9.1
Australia	Chandler	8.2	0.2	159.3	16.2	183.8
	Fernette	6.6	0.8	164.1	17.7	189.1
	Fernor	5.5	0.9	116.9	12.4	135.6
	Howard	6.7	1.2	136.6	11.7	156.2
	Lara	6.0	nd	155.7	25.1	186.7
	Serr	4.9	0.4	123.4	24.0	152.5
	Vina	5.7	nd	125.1	25.6	156.5
	Mean	6.2 $\pm$ 0.4	0.5 $\pm$ 0.2	134.9 $\pm$ 6.2	18.3 $\pm$ 2.4	159.9 $\pm$ 7.1

\*Results are mean values of duplicate determinations. nd = not detected.

## Chapter 5

### Discussion

#### 5.1 Organoleptic Analysis

##### 5.1.1 Initial assessment

In the initial assessment of the walnuts it can be seen that many of the evaluated parameters were physical parameters such as attractiveness, shell seal, crackout score and shell shape (Table 4.1). Flavour testing only formed a small part of this evaluation. It is important to note that nearly all the assessment parameters used in this evaluation are what could be described as subjective results. Shell attractiveness, ease of extraction, kernel shape, kernel flavour and kernel colour are all measures that were given a mark out of 20 or 10 based on the subjective opinion of the small group of evaluators.

Improvements could be made to remove the subjectiveness from this assessment method and to improve the method of scoring. For instance, in this method, flavour is only given a 10% weighting in the overall score. However other factors such as shell seal and attractiveness are important features for a commercial cultivar to be successful, and should not be ignored.

A more accurate crackout score was performed (Table 4.5). It can be clearly seen this provided increasingly more accurate information compared to the initial assessment, where all of the Australian walnut were given a score of 20/20 and the New Zealand walnuts had scores that ranged from 0 to 20.

There is not any literature available to compare the crackout scores to, as this is an industry specific measurement and is not normally scientifically measured and reported.

##### 5.1.2 Organoleptic assessment

Previous researchers using untrained panels to evaluate walnuts have used 51 members (Mexis *et al.*, 2009; Mexis and Kontominas, 2009) and 40 non-experts (Colarič *et al.*, 2006).

Other sensory research on walnuts has involved the descriptive analysis of walnuts. An in-depth study of the differences in the sensory characteristics between walnuts from different countries, used 7 highly trained assessors (Sinesio and Moneta, 1997).

Warmund *et al.* (2009) used 8 highly trained female panellists to perform a descriptive analysis between Black and Persian walnuts. Seven trained panellists were used by Jensen *et al.* (2003) to evaluate differences between walnuts packaged differently.

Overall there was no significant difference between the overall New Zealand versus Australian mean scores of 3.2 and 3.1 respectively for taste and mean scores of 2.9 and 2.9 respectively for after taste (Table 4.2). This was done using a ANVOA of the means at a 90% level of confidence.

Table 4.2 shows the individual cultivar means. An ANOVA was performed testing each individual cultivar mean to each other, to determine any statistical differences. The level of confidence chosen was 90%, as when the test was performed at 95 or 99% there were no clear differences.

The taste score significant differences (Table 4.3) show no one cultivar was predominately different to another, although there were some individual differences in scores. Serr (Australia) (2.8), has the most significant differences, being similar only to Meyric (2.8), Rex (3.1), Serr(New Zealand)(3.1) and Fernor (2.9).

For the after taste score significant differences (Table 4.4), Fernette (3.5) was significantly different than all of the other cultivars, and Serr (Australia) (2.4) was significantly different to all other cultivars apart from Meyric (2.8) and Vina (Australia) (2.7).

Colarič *et al.* (2006) performed a sensory evaluation on 10 different walnut cultivars grown in Maribor, Slovenia, which included the cultivars, Chandler, Fernette, Fernor and Lara. The focus of their experiment was not entirely on walnut cultivar difference, but on panellists performance as well, hence some of their results are not entirely comparable to the results of this experiment.

In their evaluation they used 6 taste descriptors; flavour, bitterness, astringency, texture, crispness and oiliness.

For flavour they found the flavour was very homogenous, with no single cultivar having a predominantly “typical walnut flavour“ (the anchor descriptor for flavour). This is comparable to our results for taste, where no single cultivar had a significantly higher taste score (anchor=flavoursome-very pleasant). However Serr (Australia) does have a significantly lower score compared to some other cultivars.

The cultivars Lara and Fernette were identified as least bitter by one of the evaluation groups in the study of Colarič *et al.* (2006) and Fernette and Fernor were among the least astringent.

It could be argued astringency and bitterness are related to the after taste of a walnut. The descriptor “after taste” is used in this experiment. Therefore Fernette having a significantly better after taste score in this experiment compares well to the findings of Colarič *et al.* (2006).

An alternative method of presenting the organoleptic results is to plot the whole set of data as a boxplot. Figures 4.1 and 4.2 show boxplots for the taste and after taste score results respectively.

Interpretations of this type of data representations, are less rigid. Boxplots are used to show trends and patterns in the data.

Of interest is the comparison of the same cultivars (Serr and Vina) grown in two different locations (New Zealand and Australia). Statistical comparison of the taste attribute means (Table 4.3), at a 90% level of confidence, showed no significant differences between Serr New Zealand (3.1) and Serr Australia (2.8) and Vina New Zealand (3.3) and Vina Australia (3.1).

Viewing the taste data as a boxplot (Figure 4.1), this shows no apparent distribution differences between the Serr (New Zealand) and Serr (Australia), however the Vina (New Zealand) would appear to be skewed more to a taste score above 3 compared to Vina (Australia).

Statistical comparison of the after taste attribute means, at 90% level of confidence, show Serr (Australia) (2.4) is significantly different to Serr (New Zealand) (3.1). There was no significant difference (Table 4.4) between the after taste means of Vina (New Zealand) (3.0) and Vina (Australia) (2.7). This trend is clearly shown again in the boxplots in Figure 4.2 . Serr (Australia) (2.4) shows a distribution that is skewed to below the mean (2.9).

This experiment clearly shows that there are differences in the taste and after taste of walnuts of the same cultivar, that are grown in a different location. The sensory test used to evaluate the walnuts is a subjective test measured using a hedonic scale. The strength of this test is its simplicity and its application to both trained and untrained panellists.

If a taste survey does not use the most basic scale anchor words of either like or dislike, the choice of anchor words is crucial. The use of anchor words other than like or dislike is

accepted, but any training or coaching is not allowed, as this will then change the type of sensory test been applied. This feature of hedonic testing has been considered when the standard was originally drafted, “The subject is allowed, however to make his own inferences about the meaning of the scale categories and determine for himself how he will apply them to the samples” (ASTM, 1968). Taking all of this into consideration the choice of flavoursome – very pleasant, to anchor the positive, high scoring end of the scale, was done as these are general words yet at the same time apply specifically to a positive walnut taste or after taste. At the other end of the scale, tasteless – bland, was chosen to anchor the more negative end, for the same reasons. However this does not preclude the fact that some people may actually like bland tasting nuts.

As mentioned, hedonic scales can be used by both experts and un-trained panellists. The majority of the panellists who tasted the walnuts in this experiment could be described as experts. This, however, is not necessarily a good thing. There is evidence to suggest that the best results from a hedonic evaluation is obtained from a untrained, non-expert panel (ASTM, 1968).

## **5.2 Biochemical analysis**

### **5.2.1 Proximate composition**

The biochemical and chemical composition of walnuts, in general has been well studied, however the composition of properly named different cultivars of walnuts has not occurred until relatively recently.

Ruggeri *et al.* (1998) is the earliest journal reference concerning the chemical composition of named walnut cultivars. In this study the proximate analysis, soluble sugar content and amino acid content of the walnut cultivars Sorrento, Franquette, Hartley and Trento were reported. The context of this research was the comparison of different walnut cultivars to other tree nut crops grown in Italy. This theme of research is common when walnuts are studied.

The general proximate analysis of 12 different cultivars of walnuts grown in New Zealand is reported by Savage (2001).

The crude protein ranged between 13.6 to 18.1, the total lipid 62.6 to 70.3, ash 1.9 to 2.4, starch 1.5 to 2.8, dietary fiber 3.1 to 5.2, ADF 2.2 to 3.8 and NDF 3.3 to 4.4 g /100g DM walnut.

This range of proximate measurements included 5 cultivars grown in New Zealand of European and United States origin. All these values are comparable to values quoted by Ruggeri *et al* (1998). No similar studies specifically on Australian grown cultivars have been performed.

### 5.2.2 Fatty acid profile

The fatty acid profile of the walnuts (Table 4.9) shows no unusual observations compared to previous literature reports (Savage *et al.*, 1999). The mean values of the Australian compared to the New Zealand fatty acids reported have nearly identical values (Table 4.9).

### 5.2.3 Tocopherol

Alpha,  $\beta$ ,  $\gamma$  and  $\delta$  tocopherols have been identified in walnuts from around the world (Amaral *et al.*, 2005; Crews *et al.*, 2005; Li *et al.*, 2007, Savage, 2000). No tocotrienols have been detected in walnuts from New Zealand (Savage, 2000). Crews *et al.* (2005) did not detect any tocotrienols in authentic walnut oils from China, France, Hungary, India, Italy, Spain and USA.

Amaral *et al.* (2005) studied 9 different cultivars from 2 different geographical locations in Portugal and found  $\gamma$ -tocotrienol in all samples. The amounts ranged from 2.25 to 5.14 mg/kg compared to total tocopherols in walnuts ranging from 194 to 632 mg/kg.

No tocotrienols were detected in this experiment from either New Zealand or Australian grown walnuts (Table 4.8).

The small amount of tocotrienols present compared to the total tocopherols and the fact Amaral *et al.* (2005) detected tocotrienols across a range of different cultivars, suggests other researchers have perhaps not used an analytical method sensitive enough.

Amaral *et al.* (2005) compared the tocopherol content of walnuts from 2 geographical locations in the same country (Portugal) and found differences in tocopherols content, however these were not significant. Significant differences were observed when cultivars were grouped by year of production, indicating differences were not only due to genetic factors but influenced by environmental factors as well.

### 5.2.4 Phytosterol

Phytosterols are not commonly analysed for in walnuts. Several researchers have analysed walnut oils for phytosterols (Martinez *et al.*, 2006; Schwartz *et al.*, 2008; Vanhanen *et al.*, 2005). Since sterols are only found in the lipid fraction it is valid to use such data for comparison.

The total phytosterol content of walnuts has been reported to range from 1,060 mg/kg – 1,770 mg/kg by Philips *et al.* (2005), 2,030 to 2,855 mg/kg by Savage *et al.* (2001) from 12 different New Zealand cultivars, 1,129.5 Maguire *et al.* (2004) and 2,340 mg/kg by Schwartz (2008). This indicates there is a lot of variability in phytosterol amounts in walnuts grown around the world. It is not known if this is due to differences in geographical locations, climate or genotype differences

In all the literature the most predominate phytosterol is  $\beta$ -sitosterol, but similarly the percentage contribution has a large range, 65%, 78% 66% and 62% of the total sterols.

Again, no conclusions can be made about the variation in the  $\beta$ -sitosterol content as there have been no studies performed specifically addressing this.

### 5.2.5 Phenols

Phenols in walnuts could be classified as a plant phytochemical (Chen and Blumberg, 2008), as are phytosterols, carotenoids and other groups of compounds that may effect a person's health either positively or negatively.

Phenols in plant derived foods are a very diverse groups of compounds. To date it is estimated that more than 6,000 different types of phenols are to be found in plants. Phenolics can be found in all parts of a food, for example, the shell, leaf and kernel of a walnut. With varying amounts and types of different phenols from different parts of the walnut.

Based on their fundamental chemical structure, phenols can be divided into two major groups called non-flavonoids and flavonoids. Within these two major groups there are many different sub-groups.

Very little , if not any, research has been done to identify and characterise walnut phenols.

Liu *et al.* (2008) has identified 3 diarylheptanoids and an  $\alpha$ -tetralone in green walnut husks of an unspecified cultivar. Oliveira *et al.* (2008) has reported the total phenolic content of aqueous extracts of green walnut husks from 5 cultivars found in Portugal.



Pereira *et al.* (2007) has reported the phenolic profile of walnut leaves. Identifying 10 aqueously extracted phenolic compounds from 6 different walnut cultivars grown in Portugal. It is important to note that walnut green husks and leaves are not normally eaten.

Researchers have reported the total phenolic content of walnut kernels, however most of the studies are not specific for walnut cultivars and are done as a consequence of surveying a range of nuts or similar food types (Arcan and Yemenicioğlu, 2009; Chen and Blumberg, 2008; Gunduc and El, 2003; Kornsteiner *et al.* 2006). Arcan and Yemenicioğlu (2009) reported a range of 175 to 414 mg gallic acid/100g dry weight walnuts. Gunduc and El (2003) reported 7,051.74 mg catechin /kg walnut. Chen and Blumberg (2008) and Kornsteiner *et al.* (2006) both report similar amounts, 16.3 mg gallic acid equivalents/g and 1,625 gallic acid equivalents /100 g fresh weight walnuts, respectively. In both Chen and Blumberg (2008) and Kornsteiner *et al.* (2006) walnuts have the highest total phenolic content compared to all the other nuts in their studies (almonds, brazil nuts, cashew, hazelnuts, macadamias, peanuts, pecans, pine nuts and pistachios).

Pereira *et al.* (2008) analysed six different cultivars of walnuts grown in Portugal, values ranged from 60.83 to 95.06 mg gallic acid equivalents/g walnut aqueous extract.

The total phenolic content in the New Zealand and Australian walnut kernels analysed in this study, ranged from a low of 14.0 (Serr, New Zealand ) to a high of 24.7 (Vina, New Zealand) mg gallic acid equivalents /g dry weight. Compared to previous research, results range from 175 to 9,506 mg gallic acid equivalents / 100g walnut kernel. With such a large range in values it is difficult to make a direct comparison and more work needs to be done in this area to make a proper evaluation.

There is no published data on the phenolic profile or total phenolic content of different walnut cultivar kernels grown in New Zealand or Australia.

To the authors knowledge this is the first report of the free and total phenolic content for named walnut cultivars grown in New Zealand and Australia.

The bioactive compounds in walnuts are normally expressed on a per 100 g of nut oil basis. This enables the comparison to previous data published in literature and if the study involves oil this is relevant.

A more relevant way is to express the walnut bioactive compounds in this study, is to use a per gram dry weight basis, to allow a whole walnut to walnut comparison.

Table 5.1, shows the total phenols , total tocopherols and total phytosterols expressed in this manner. The total phenols were originally calculated this way.

As can be seen in Table 5.1 there are inconsistencies in the New Zealand walnuts in identifying the highest and lowest total tocopherols values, depending on how they are expressed and for identifying the highest total phytosterol values in Australian walnuts.

Table 5.1 Mean total phenolic, tocopherol and phytosterol content per gram dry weight of walnut kernel in New Zealand (Canterbury) and Australian (Tasmanian) grown walnuts.

	Cultivar	Total phenols mg GAE <sup>*</sup> /g	Total tocopherols µg/g	Total Phytosterols µg/g
New Zealand	Earnsleugh	15.7	250.9	1,044.9
	Kirwee	18.6	260.1 <sup>H</sup>	1,010.1 <sup>L, Lo</sup>
	Meyric	17.9	255.5	1,222.2
	Rex	16.0	255.0 <sup>Ho</sup>	1,319.7
	Serr	14.0 <sup>L</sup>	239.2 <sup>Lo</sup>	1,387.4 <sup>H, Ho</sup>
	Vina	24.7 <sup>H</sup>	236.1 <sup>L</sup>	1,010.7
	Mean (±SE)	17.5 ± 1.5	249.5 ± 3.9	1,165.8 ± 68.0
Australia	Chandler	15.6	238.6	1,218.5
	Fernette	14.8	237.4	1,305.1 <sup>Ho</sup>
	Fernor	23.6 <sup>H</sup>	243.2	939.62 <sup>L, Lo</sup>
	Howard	19.5	269.9 <sup>H, Ho</sup>	1,048.8
	Lara	14.4 <sup>L</sup>	257.6	1,325.1 <sup>H</sup>
	Serr	16.1	215.0 <sup>L, Lo</sup>	1,082.3
	Vina	19.5	231.5	1,050.2
	Mean (±SE)	17.5 ± 1.3	241.9 ± 6.7	1,138.5 ± 55.1

\* = gallic acid equivalents.

<sup>H</sup> = highest and <sup>L</sup> = lowest value on a per gram dry weight basis. <sup>Ho</sup> = highest and <sup>Lo</sup> = lowest value on a per 100 mL oil basis.

### 5.3 Correlation between organoleptic and biochemical results

The organoleptic results concluded there was no differentiation between Serr and Vina varieties grown in Australia and New Zealand, this too was reflected in the biochemical results (Tables 4.6 to 4.10).

The organoleptic results identified Fernette (Australia) as performing best under test conditions for the descriptors flavour and after taste.

Comparing Fernette to individual biochemical results shows no difference to most of the other walnut cultivars.

The organoleptic results and biochemical results were also compared statistically using a Pearson correlation (Minitab, data not shown). There were no significant correlations between both the organoleptic attributes, taste and after taste, when correlated to any of the biochemical results.

Using total phenols as an example, as phenolic compounds could be expected to have an influence on the taste parameters. The Pearson correlation between total phenols and taste attribute is -0.184, p-value= 0.547 and between total phenols and after taste attribute is -0.257, p-value = 0.398.

## Chapter 6

### Conclusions

#### 6.1 Organoleptic Analysis

There is no significant difference between the overall mean taste and after taste scores between walnuts grown in Canterbury, New Zealand and Tasmania, Australia.

Fernette, grown in Tasmania, Australia, had the best mean taste score (3.5), which was significantly different to some of the other walnuts grown in New Zealand and Australia.

Fernette had the best after taste score (3.5) which was significantly different to all other walnuts.

Comparison of Serr and Vina grown in both New Zealand and Australia showed no significant difference between the mean taste scores of the same cultivars, however a boxplot clearly exhibits a skew in the data to Vina New Zealand for a better taste score.

The mean after taste score of Serr (New Zealand) compared to Serr (Australia) was significantly different, this was supported by visually skewed data for Serr (Australia), to a lower after taste score.

There is evidence from our study and others (Colarič *et al.*, 2006) that suggests Fernette performs well using the descriptors flavour and after taste.

It should be remembered that the selection of potential new cultivars will also involve an assessment of the trees response to the local environment

#### 6.2 Biochemical analysis

There were no conclusive differences between Australian and New Zealand grown walnuts when comparing the proximate analysis, fatty acid profile, tocopherol profile, phytosterol profile or phenolic analysis.

Of interest is the phytosterol and, total and free phenolic analysis of the walnuts. Walnut phytosterol content has not been widely reported, similarly for the total and free phenolic content of walnuts, both these parameters warrant further investigation.

### 6.3 Future research

The results from this study provided valuable information for the NZWIG to make decisions regarding the importation of new cultivars that are potentially beneficial to the New Zealand walnut industry. This research has also advanced the body of knowledge with regards to inter-cultivar differences of different walnut cultivars.

There are areas that could be explored in the future, these are:

- The further development of a New Zealand in-shell and walnut kernel quality standard that is recognised internationally, as well as by the appropriate governing bodies.
- Previous phenolic research has focused on the total phenolic content not the free. This is a new and recent concept in the field of food science. There is ambiguity surrounding the use of the terms “free” and “total” in this context, additionally attention and care needs to be taken as to whether the results are quoted on a dry or wet basis, as cited research is often ambiguous on this point too.
- The phenolic profiling work on walnuts so far, has not been done directly on the walnut kernel. Researchers have identified the phenolic compounds in walnut husk, walnut oil and walnut flour, the assumption being the same phenols are found in a fresh walnut kernel, as we would eat it, this may not be the case.

## Chapter 7

### References

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## Chapter 8

### Appendix A.1

#### **STANDARD 2.3.1**

#### **FRUIT AND VEGETABLES**

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##### **Purpose**

This Standard provides specific definitions for fruit and vegetables, which include nuts, spices, herbs, fungi, legumes and seeds based on processing categories in Standard 1.3.1 for the purpose of providing specific additive permissions.

##### **Table of Provisions**

- 1 Interpretation
- 2 Composition

##### **Clauses**

- 1 Interpretation

In this Code -

**fruit and vegetables** means fruit, vegetables, nuts, spices, herbs, fungi, legumes and seeds.

**peeled and/or cut fruit and vegetables** means fruit and vegetables that are peeled and/or cut, whether or not they have been surface treated.

**surface treated fruit and vegetables** means fruit and vegetables harvested, washed and treated with permitted processing aids and food additives.

- 2 Composition

Fruit and vegetables in brine, oil, vinegar or water, other than commercially canned fruit and vegetables, must not have a pH greater than 4.6.

## Appendix A.2

UNITED  
NATIONS

**E**



**Economic and Social  
Council**

Distr.  
GENERAL

ECE/TRADE/C/WP.7/2008/16  
21 August 2008

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**ECONOMIC COMMISSION FOR EUROPE**

**COMMITTEE ON TRADE**

Working Party on Agricultural Quality Standards

Sixty-fourth session  
Geneva, 3-6 November 2008  
Item 5(b) of the provisional agenda

**TEXTS FOR ADOPTION AS NEW/REVISED UNECE STANDARDS**

**Inshell Walnuts**

Note by the secretariat

This text is submitted to the Working Party for approval as a revised Standard for Inshell Walnuts.

It is based on document TRADE/WP.7/2002/9/Add.14 and includes changes to the standard layout adopted at the fifty-ninth session (November, 2003) of the Working Party of the Working Party on Agricultural Quality Standards.

GE.08-25093

**UNECE STANDARD DDP-01**  
concerning the marketing and commercial quality control of

**INSHELL WALNUTS**

**I. DEFINITION OF PRODUCE**

This standard applies to inshell walnuts free from outer husks, from varieties (cultivars) grown from *Juglans regia* L., to be supplied to the consumer, walnuts for making oil or to be cracked for extraction of the kernels being excluded.

The expression "fresh walnuts" or "early walnuts" means walnuts which have been marketed soon after harvesting and cannot be preserved for a long time, from which the husk has been removed and which have not been treated in any way that tends to change their natural moisture content.

The expression "dry walnuts" means walnuts which can be preserved for a long time under normal conditions of storage.<sup>1</sup>

**II. PROVISIONS CONCERNING QUALITY**

The purpose of the standard is to define the quality requirements of inshell walnuts at the export-control stage, after preparation and packaging.

**A. Minimum requirements<sup>2</sup>**

**(i) General provisions**

In all classes, subject to the special provisions for each class and the tolerances allowed, the inshell walnuts must be:

**(a) Characteristics of the shell**

- intact;
- slight superficial damage is not considered as a defect;
- partially open walnuts are considered to be intact provided that the kernel is physically protected;
- sound;
- free from defects likely to affect the natural keeping quality of the fruit;
- free from attack by pests;
- clean; practically free of any visible foreign matter;
- dry; free from abnormal external moisture;
- free from husks.

<sup>1</sup> In the case of transport by closed container, special attention should be paid to air circulation in the container and to the moisture content of the produce.

<sup>2</sup> The definition of defects is given in the Annex to this document.

The shells of dry walnuts must show no trace of hulling.

**(b) Characteristics of the kernels**

- sound; produce affected by rotting or deterioration such as to make it unfit for consumption is excluded;
- firm;
- clean, practically free from any visible foreign matter;
- free from living insects or mites whatever their stage of development;
- free from visible damage by insects, mites or other parasites;
- free from rancidity and/or oily appearance;
- free from mould;
- free of abnormal external moisture;
- free of foreign smell and/or taste;
- normally developed, shrivelled kernels are to be excluded.

**(c) The inshell walnuts must be gathered when sufficiently mature.**

Walnuts must not be empty.

In the case of "fresh walnuts", it must be possible to peel off the skin of the kernel easily and the internal central partition must show signs of turning brown.

In the case of "dry walnuts", the internal central partition must be dry.

The shells may be washed and bleached provided that the treatment applied does not affect the quality of the kernels and is permitted by the regulations of the importing country.

The condition of the inshell walnuts should be such as to enable them:

- to withstand transportation and handling
- to arrive in a satisfactory condition at the place of destination.

(ii) *Moisture content*<sup>3</sup>

The dry walnuts shall have a moisture content not exceeding 12 per cent for the whole nut and 8 per cent for the kernel.<sup>4</sup>

The natural moisture content of whole fresh walnuts shall be equal to or greater than 20 per cent.

**B. Classification**

In accordance with the defects allowed in section "IV. Provisions concerning tolerances", inshell walnuts are classified into the following classes:

(i) *"Extra" class*

Inshell walnuts in this class must be of superior quality. They must have the characteristics of the variety or of the mixture of certain varieties officially defined by the producing country and specified in the marking.

They must be practically free from defects with the exception of very slight superficial defects provided that these do not affect the general appearance of the produce, the quality, the keeping quality or its presentation in the package.

This class may not include inshell walnuts whose variety cannot be guaranteed, nor a mixture which is not defined.

Furthermore, only walnuts of the most recent harvest can be included in this class.

(ii) *Class I*

Inshell walnuts in this class must be of good quality. They must embody the characteristics of the variety, of a commercial type or of a mixture of certain varieties officially defined by the producing country and specified in the marking.

Slight defects may be allowed provided that these do not affect the general appearance of the produce, the quality, the keeping quality or its presentation in the package.

<sup>3</sup> The moisture content is determined by one of the methods given in annex II of the Standard Layout – Determination of the moisture content for dry produce  
<[http://www.unctad.org/trade/agr/standards/dry/Standard\\_layout/Standard\\_layoutDDP\\_e.pdf](http://www.unctad.org/trade/agr/standards/dry/Standard_layout/Standard_layoutDDP_e.pdf)>. The laboratory reference method shall be used in cases of dispute.

<sup>4</sup> The method to be used should be one of the methods tested collaboratively and shown to give satisfactory results in inter-laboratory trials for the determination of the moisture content for dry fruit (inshell nuts and kernels) which are given in the standard layout and are reproduced in the annex to this document. The laboratory reference method shall be used in cases of dispute.

<sup>5</sup> Reservation from Romania, requesting 10% and 6% respectively.

This class may not include inshell walnuts whose variety cannot be guaranteed, nor a mixture which is not defined.

(iii) *Class II*

This class comprises inshell walnuts which do not qualify for inclusion in the higher classes, but satisfy the minimum requirements specified above.

Defects may be allowed provided that the inshell walnuts retain their essential characteristics as regards general appearance, quality, keeping quality and presentation.

**III. PROVISIONS CONCERNING SIZING**

Size is defined either by an interval determined by the minimum diameter and the maximum diameter (sizing) or by an indication of the minimum diameter followed by "and above" or "and +" (screening).

Class	Sizing <sup>a</sup>	Screening <sup>a</sup>
Extra and I	32 to 34 mm	34 mm and above
	30 to 32 mm	32 mm and above
	28 to 30 mm	30 mm and above
	27 to 30 mm for oblong varieties <sup>c</sup>	28 mm and above <sup>b</sup> 27 mm and above for oblong varieties <sup>b,c</sup>
II	24 to 28 mm	24 mm and above
	24 to 27 mm for oblong varieties <sup>c</sup>	

<sup>a</sup> In addition to this sizing and screening table, provided that the size is also expressed in the marking, any size name may be used optionally.

<sup>b</sup> Products classified in Class I may exceptionally be marketed with a screening of 26mm and above.

<sup>c</sup> The varieties of oblong walnuts have a shell whose height is at least 1.25 times the maximum diameter of the equatorial section.

**IV. PROVISIONS CONCERNING TOLERANCES**

Tolerances in respect of quality and size shall be allowed in each package for produce not satisfying the requirements of the class indicated.

#### A. Quality tolerances

In the calculation of tolerances, whatever the class, two half-empty walnuts or four quarter-empty walnuts are counted as one empty walnut.

Defects allowed <sup>a</sup>	Tolerances allowed (percentage by number of defective fruit)		
	Extra	Class I	Class II
(a) Total tolerances for defects of the shell	7	10	15
(b) Total tolerances for defects of the edible part <sup>b</sup>	8	10	15
of which rancid, rotten or damaged by insects <sup>c</sup>	3	6	8
of which mouldy walnuts	3	4	6

<sup>a</sup> The definitions of defects are listed in the Annex to this document.

<sup>b</sup> For fresh walnuts, the tolerances for defects of the kernel are as follows: "Extra" Class: 8%; Class I: 12%; Class II: 15%.

<sup>c</sup> Living insects or animal pests are not permitted in any class.

#### B. Mineral impurities

Ashes insoluble in acid must not exceed 1 g/kg.

#### C. Size tolerances

For all classes, a maximum of 10 per cent of inshell walnuts not conforming to the size indicated in the marking is tolerated within the limits such that:

- the nuts correspond to the sizes immediately below or above when the size is designated by an interval determined by the minimum diameter and the maximum diameter (sizing);
- the nuts correspond to the size immediately below when the size is designated by an indication of the minimum diameter followed by "and above" or "and +" (screening).

### V. PROVISIONS CONCERNING PRESENTATION

#### A. Uniformity

The contents of each package must be uniform and contain only inshell walnuts of the same origin, crop year and quality. In a single package stated to contain a given variety, a defined mixture of varieties or commercial type, a maximum of 10 per cent of inshell walnuts may belong to other varieties or commercial types.

The visible part of the package must be representative of the entire contents.

#### B. Packaging

Inshell walnuts must be packed in such a way as to protect the produce properly.

The materials used inside the package must be new, clean and of a quality such as to avoid causing any external or internal damage to the produce. The use of materials, particularly of paper and stamps bearing trade specifications, is allowed provided that the printing or labelling has been done with a non-toxic ink or glue.

Packages must be free of all foreign matter.

#### C. Presentation

The weight of the packages constituting a batch must be identical. <sup>4</sup>

### VI. PROVISIONS CONCERNING MARKING

Each package<sup>7</sup> must bear the following particulars in letters grouped on the same side, legibly and indelibly marked and visible from the outside:

#### A. Identification

Packer	)	Name and address or	<sup>8</sup>
and/or	)	officially issued or	
Dispatcher	)	accepted code mark <sup>4</sup>	

<sup>4</sup> The regulations of certain importing countries require compliance with a specific range of net weights for closed packages.

<sup>7</sup> Package units of produce prepacked for direct sale to the consumer shall not be subject to these marking provisions, but shall conform to national requirements. However, the markings referred to shall in any event be shown on the transport packaging containing such package units.

<sup>8</sup> The national legislation of a number of countries requires the explicit declaration of the name and address. In cases where a code mark is used, the reference "packer and/or dispatcher" (or equivalent abbreviations) must be indicated in close connection with the code mark.



**B. Nature of produce**

- "Fresh walnuts" or "Early walnuts" (in the case of fresh walnuts)
- "Walnuts" or "Dry walnuts" (in the case of dry walnuts);
- Name of the variety or of the mixture defined for the "Extra" class; name of the variety, defined mixture or commercial type for class I.

**C. Origin of produce**

- Country of origin and, optionally, district where grown or national, regional or local place name.

**D. Commercial specifications**

- Class;
- Size, which may be given either:
  - by the minimum and maximum diameters, or
  - by the minimum diameter followed by the words "and above" or "and +";
- Crop year (mandatory for "Extra" class and class I, optional for class II);
- Net weight;
- Date of packaging mandatory for fresh walnuts and optional for dry walnuts;
- Best before followed by the date (optional); for fresh walnuts the indication "To be consumed quickly, store preferably in a cool place", or the indication "Preservation very limited, store preferably in a cool place."

**E. Official control mark (optional)**

This standard was first published as UNECE Standard for Unshelled Walnuts in 1970  
Revised 1983  
Partially Revised 1991 (Standard Layout)  
Revised and adopted as UNECE Standard for Inshell Walnuts 1999  
Editorial revision and inclusion of new Annex I 2002

**ANNEX I: DETERMINATION OF THE MOISTURE CONTENT FOR DRY PRODUCE (NUTS)**

Based on Annex II of the Standard Layout

**METHOD 1 - LABORATORY REFERENCE METHOD**

**1. Scope and application**

This reference method serves to determine the moisture and volatile matter content for both inshell nuts and shelled nuts (kernels).

**2. Reference**

This method is based on the method prescribed by ISO: ISO 665-2000 Oilseeds - Determination of moisture and volatile matter content.

**3. Definition**

Moisture content and volatile matter content for dry produce (inshell nuts and shelled nuts): loss in mass measured under the operating conditions specified in ISO 665-2000 for oilseeds of medium size (see point 7.3 of ISO 665-2000). The moisture content is expressed as mass fraction, in percent, of the mass of the initial sample.

For whole nuts, when moisture content is expressed both on the whole nut and on the kernel, in cases of dispute between the two values, the moisture content value of the whole nut takes precedence.

**4. Principle**

Determination of the moisture and volatile matter content of a test portion by drying at  $103 \pm 2^\circ\text{C}$  in an oven at atmospheric pressure, until practically constant mass is reached.

**5. Apparatus (see ISO 665-2000 for more details)**

- 5.1 Analytical balance sensitive to 1 mg or better.
- 5.2 Mechanical mill.
- 5.3 3 mm round-holes sieve.
- 5.4 Glass, porcelain or non-corrosive metal containers, provided with well-fitting lids, allowing the test portion to be spread to about  $0.2\text{ g/cm}^2$  (approximately 5 mm height).

- 5.5 Electric oven with thermostatic control capable of being regulated between 101 and 105°C in normal operation.
- 5.6 Desiccator containing an effective desiccant.

## 6. Procedure

Follow the operating conditions as specified in ISO 665-2000 for oilseeds of medium size (point 7 and 7.3 of ISO 665-2000), but with the following specific modifications, concerning the preparation of the test sample.

Although ISO 665-2000 sets up one initial period of 3 hours in the oven set at 103 ± 2°C, for nuts it is recommended one initial period of 6 hours.

### 6.a Determination of the moisture and volatile matter content of kernels:

For shelled nuts, homogenize the laboratory sample and take a minimum of 100 g of kernels as a test sample.

For inshell nuts, take a minimum of 200 g and, using a nutcracker or hammer, remove the shells and fragments or particles of shell, using the rest as a test sample. The kernel skin (cuticle or spermoderm) is included in the test sample.

Grind and sieve the test sample until the size of the particles obtained is no greater than 3 mm. During the grinding operation, care should be taken to avoid the production of a paste (oily flour), the overheating of the sample and the consequent loss of moisture content (for example, if using a mechanical food chopper, by successive very short grinding and sieving operations).

Spread evenly over the base of the vessel about 10 g of the ground product as a test portion, replace the lid, and weigh the whole vessel. Carry out two determinations on the same test sample.

### 6.b Determination of moisture and volatile matter content on whole nuts (shell plus kernel):

Homogenize the laboratory sample and take a minimum of 200 g of nuts as a test sample. Remove all the foreign matter (dust, stickers, etc.) from the test sample.

Grind the whole nuts using either a Rass Mill, a Romer Mill or a Brabender apparatus or similar, without overheating the product.

Spread evenly over the base of the vessel about 15 g of the ground product as a test portion, replace the lid, and weigh the whole vessel. Carry out two determinations on the same test sample.

## 7. Expression of results and test report

Follow all the instructions as specified in ISO 665-2000 (point 9 and 11) for method of calculation and formulae, and for test report, without any modification.<sup>9</sup>

## 8. Precision

For conditions of repeatability and reproducibility apply specifications of ISO 665-2000 (point 10.2 and 10.3) for soya beans.

## METHOD 2: RAPID METHOD

### 1. Principle

Determination of the moisture content using a measuring apparatus based on the principle of loss of mass by heating. The apparatus should include a halogen or infra-red lamp and a built-in analytical balance, calibrated according to the laboratory method.

The use of apparatus based on the principle of electrical conductivity or resistance, as Moisture Meters, Moisture Testers and similar, is also allowed always at condition that the apparatus has to be calibrated according with the laboratory reference method for the tested product.

### 2. Apparatus

- 2.1 Mechanical mill or food chopper.
- 2.2 3 mm round-holes sieve (unless indicated otherwise by the instructions for use of the apparatus).
- 2.3 Halogen or infrared lamp with built-in analytical balance sensitive to 1 mg or better.

### 3. Procedure

#### 3.1 Preparation of sample

Follow the same instructions as given for the laboratory reference method (points 6.a and 6.b), unless indicated otherwise by the instructions for use of the apparatus, particularly with regard to the diameter of the fragments.

#### 3.2 Determination of moisture content

<sup>9</sup> The main points specified are as follows:

- moisture and volatile matter content is expressed as mass fraction, in percent, of the mass of the initial sample.
- The result is the arithmetic mean of the two determinations; the difference between the two determinations should not exceed 0.2 % (mass fraction).
- The result has to be reported to one decimal place.

Carry out the determination on two test portions of approximately 5 to 10 g each, unless indicated otherwise by the instructions for use of the apparatus.

Spread the test portion over the base of the test receptacle, thoroughly cleaned in advance, and note the weight of the test portion to within 1 mg.

Follow the procedure indicated in the instructions for use of the apparatus for the product to be tested, in particular with regard to the adjusting of temperatures, the duration of the test and the recording of the weight readings.

#### 4. Expression of results

##### 4.1 Result

The result should be the arithmetic mean of the two determinations, provided that the conditions of repeatability (4.2) are satisfied. Report the result to one decimal place.

##### 4.2 Repeatability

The difference in absolute value between the respective results of the two determinations performed simultaneously or one immediately after the other by the same operator, under the same conditions on identical test material, must not exceed 0.2%.

#### 5. Test report

The test report must state the method used and the results obtained. The report must contain all information necessary for the full identification of the sample.

### ANNEX II: DEFINITION OF DEFECTS FOR INSHELL WALNUTS Based on Annex II of the Standard Layout

#### A. Defects of the shell:

Any defect affecting the appearance including:

- staining or discolouration: abnormal colour which covers 20% of the surface of the shell of an individual nut and which is of a brown, reddish brown, grey or other colour in pronounced contrast with the colour of the rest of the shell or the majority of shells in the lot;
- adhering dirt, when affecting more than 5 % of the shell surface;
- adhering husk, when affecting more than 10% of the shell surface;
- husking damages: pronounced marks on the shell caused by the operation of mechanically removing the husk.

#### B. Defects of the edible part (kernel):

Any defects affecting the appearance of the kernel, including blemishes or areas of discolouration: discolouration which affects more than one quarter of the kernel and which is in pronounced contrast with the colour of the rest of the kernel.

Shrivelled kernels: Kernel which is seriously shrunk, wrinkled and tough.

Ripening defects for fresh nuts: Kernel which is not sufficiently firm, of which for fresh walnuts: the skin cannot easily be peeled off and/or of which the internal central partition does not show signs of turning brown.

Rancidity: Oxidation of lipids or free fatty acid production producing a disagreeable flavour.

Empty nuts: Condition of the walnut in which the kernel has not developed.

#### C. Defects of the shell and kernel

Mould: Mould filaments visible to the naked eye.  
Decay: Significant decomposition caused by the action of micro-organisms.

Insect damage: Visible damage caused by insects or other animal parasites or the presence of dead insects or insect debris.

Foreign matter: Any matter or material not usually associated with the product.  
Mineral impurities: Acid insoluble ash.

Foreign smell or taste: Any odour or flavour that is not characteristic of the product.

\* \* \* \* \*

## Appendix A.3



United States  
Department of  
Agriculture

Agricultural  
Marketing  
Service

Fruit and  
Vegetable  
Division

Fresh  
Products  
Branch

# **United States Standards for Grades of Shelled Walnuts (*Juglans regia*)**

**Effective September 1, 1968**  
*(Reprinted - January 1997)*

## United States Standards for Grades of Shelled Walnuts (*Juglans regia*)<sup>1</sup>

### General

§1.2275 Application.

§1.2276 Color chart.

### Grades

§1.2277 U.S. No. 1.

§1.2278 U.S. Commercial.

### Unclassified

§1.2279 Unclassified.

### Tolerances for Grade Defects

§1.2280 Tolerances for grade defects.

### Color Requirements

§1.2281 Color classifications.

§1.2282 Tolerances for color.

§1.2283 Off color.

### Size Requirements

§1.2284 Size classifications.

§1.2285 Tolerances for size.

### Application of Tolerances

§1.2286 Application of tolerances.

### Definitions

§1.2287 Well dried.

§1.2288 Clean.

§1.2289 Shell.

§1.2290 Insect injury.

§1.2291 Rancidity.

§1.2292 Damage.

§1.2293 Serious damage.

§1.2294 Very serious damage.

§1.2295 Half kernel.

§1.2296 Three-fourths half kernel.

### General

§1.2275 Application.

The standards contained in this subpart apply only to walnuts commonly known as English or Persian walnuts (*Juglans regia*). They do not apply to walnuts commonly known as black walnuts (*Juglans nigra*).

<sup>1</sup>Compliance with the provisions of these standards shall not excuse failure to comply with the provisions of the Federal Food, Drug, and Cosmetic Act, or with applicable State laws and regulations.

### §1.2276 Color chart.

The color chart (USDA Walnut Color Chart) to which reference is made in §§1.2281 and 1.2282 illustrates the four shades of walnut skin color listed as color classifications.

(a) **Availability of color chart.** The USDA Walnut Color Chart cited in this subpart has been filed with the original document and is available for inspection in the Office of the Federal Register. The color chart is also available for inspection in the Fruit and Vegetable Division, AMS, U.S. Department of Agriculture, South Building, Washington, D.C. 20250, in any field office of the Fresh Fruit and Vegetable Inspection Service of the Fruit and Vegetable Division, or upon request of any authorized inspector of such Service. Copies of the color chart may be purchased from Munsell Color Co., Inc., 2441 North Calvert Street, Baltimore, Md. 21218.

### Grades

§1.2277 U.S. No. 1.

"U.S. No. 1" consists of portions of walnut kernels which are well dried, clean, free from shell, foreign material, insect injury, decay, rancidity, and free from damage caused by shriveling, mold, discoloration of the meat or other means. (See §1.2280.)

(a) Color shall be specified in connection with this grade in terms of one of the color classifications. (See §§1.2276, 1.2281 and 1.2282.)

(b) Size shall be specified in connection with this grade in terms of one of the size classifications. (See §§1.2284 and 1.2285.)

§1.2278 U.S. Commercial.

"U.S. Commercial" consists of portions of walnut kernels which meet the requirements of U.S. No. 1 grade, except for increased tolerances. (See §1.2280.)

(a) Color of walnuts in this grade shall be not darker than "amber" classification, and color need not be specified. However, color may be specified in connection with the grade in terms of one of the color classifications. (See §§1.2276, 1.2281 and 1.2282.)

(b) Size shall be specified in connection with this grade in terms of one of the size classifications. (See §§1.2284 and 1.2285.)

### Unclassified

§1.2279 Unclassified.

"Unclassified" consists of portions of walnut kernels which have not been classified in accordance with either of the foregoing grades. The term "unclassified" is not a grade within the meaning of these standards, but is provided as a designation to show that no grade has been applied to the lot.

### Tolerances for Grade Defects

§1.2280 Tolerances for grade defects.

(a) All percentages shall be calculated on the basis of weight.

(b) In order to allow for variations, other than for color and size, incident to proper grading and handling, tolerances shall be permitted for the respective grades as indicated in Table I:

Table I

Grade	Tolerances for grade defects			
	Total defects	Serious damage	Very serious damage	Shell and foreign material
	Percent	Percent	Percent	Percent
U.S. No. 1.....	5	2 (included in 5 percent total defects).	1 (included in 2 percent serious damage).	0.05 (included in 1 percent very serious damage).
U.S. Commercial.....	8	4 (included in 8 percent total defects).	2 (included in 4 percent serious damage).	0.05 (included in 2 percent very serious damage).

**Color Requirements****§51.2281 Color classifications.**

The following classifications are provided to describe the color of any lot: "Extra Light", "Light", "Light Amber" or "Amber". The portions of kernels in the lot shall not be darker than the darkest color permitted in the specified classification as shown on the color chart.

**§51.2282 Tolerances for color.**

(a) All percentages shall be calculated on the basis of weight.

(b) In order to allow for variations incident to proper grading and handling, tolerances shall be permitted for the respective color classifications as indicated in Table II:

Table II

Color classification	Tolerances for color			
	Darker than extra light <sup>1</sup>	Darker than light <sup>1</sup>	Darker than light amber <sup>1</sup>	Darker than amber <sup>1</sup>
Extra light.....	15 percent	2 percent (included in 15 percent darker than extra light).	-----	-----
Light.....	-----	15 percent	2 percent (included in 15 percent darker than light).	-----
Light amber.....	-----	-----	15 percent	2 percent (included in 15 percent darker than light amber).
Amber.....	-----	-----	-----	10 percent.

<sup>1</sup>See illustration of this term on color chart.

**§51.2283 Off color.**

The term "off color" is not a color classification, but shall be applied to any lot which fails to meet the requirements of the "Amber" classification.

**Size Requirements****§51.2284 Size classification.**

The following classifications are provided to describe the size of any lot: "Halves", "Pieces and Halves", "Pieces" or "Small Piece". The size of portions of kernels in the lot shall conform to the requirements of the specified classification as defined below:

(a) **Halves.** Lot consists of 85 percent or more, by weight, half kernels, and the remainder three-fourths half kernels. (See §51.2285.)

(b) **Pieces and halves.** Lot consists of 20 percent or more, by weight, half kernels, and the remainder portions of kernels that cannot pass through a sieve with 24/64 inch round openings. When a lot exceeds this minimum requirement, the actual percentage of halves may be specified. (See §51.2285.)

(c) **Pieces.** Lot consists of portions of kernels that cannot pass through a sieve with 24/64 inch round openings. (See §51.2285.)

(d) **Small pieces.** Lot consists of portions of kernels that pass through a sieve with 24/64 inch round openings, but that cannot pass through a sieve with 8/64 inch round openings. When desired, the actual size ranges within such size ranges may be specified. (See §51.2285.)

**§51.2285 Tolerances for size.**

(a) All percentages shall be calculated on the basis of weight.

(b) In order to allow for variations incident to proper sizing and handling, tolerances shall be permitted for the respective size classifications as indicated in Table III:

Table III

Size classification	Tolerances for size				
	Smaller than three-fourths halves	Will not pass through 24/64 inch round hole	Pass through 24/64 inch round hole	Pass through 16/64 inch round hole	Pass through 8/64 inch round hole
	Percent	Percent	Percent	Percent	Percent
Halves.....	5	-----	-----	1 (included in 5 percent).	-----
Pieces and halves <sup>1</sup>	-----	-----	18	3 (included in 18 percent).	1 (included in 3 percent).
Pieces.....	-----	-----	25	5 (included in 25 percent).	1 (included in 5 percent).
Small pieces <sup>2</sup> .....	-----	10	-----	-----	2.

<sup>1</sup>No part of any tolerance shall be used to reduce the percentage of halves required or specified in a lot of "pieces and halves."

<sup>2</sup>The tolerances of 10 percent and 2 percent for "small pieces" classification shall apply, respectively, to any smaller maximum or any larger minimum sizes specified.

**Application of Tolerances****§51.2286 Application of tolerances.**

The tolerances provided in these standards are on a lot basis, and they shall be applied to a

composite sample representative of the lot. However, any container or group of containers in which the walnuts are obviously of a quality materially different from that in the majority of containers shall be considered a separate lot, and shall be sampled separately.

#### Definitions

##### §51.2287 Well dried.

"Well dried" means that the portion of kernel is firm and crisp, not pliable or leathery.

##### §51.2288 Clean.

"Clean" means that the appearance of the individual portion of kernel, or of the lot as a whole, is not materially affected by adhering dust, dirt or other foreign material.

##### §51.2289 Shell.

"Shell" means the outer shell and/or the woody partition from between the halves of the kernel, and any fragments of either.

##### §51.2290 Insect injury.

"Insect injury" means that the insect, web, frass or other evidence of insects is present on the portion of kernel.

##### §51.2291 Rancidity.

"Rancidity" means that the portion of kernel is noticeably rancid to the taste. Rancidity should not be confused with a slightly astringent flavor of the pellicle (skin) or with staleness (the stage at which the flavor is flat but not objectionable).

##### §51.2292 Damage.

"Damage" means any defect, other than color, which materially affects the appearance, or the edible or shipping quality of the individual portion of kernel, or of the lot as a whole. Any one of the following defects or any combination of defects the seriousness of which exceeds the maximum allowed for any one defect shall be considered as damage:

(a) Shriveling when more than one-eighth of the portion of kernel is severely shriveled, or a greater area is affected by lesser degrees of shriveling producing an equally objectionable appearance, except that kernels which are thin in cross-section but which are otherwise normally developed shall not be considered as damaged;

(b) Mold when plainly visible;

(c) Discoloration of the meat when more than one-eighth the volume of the portion of kernel is severely discolored, or a greater volume is affected by lesser degrees of discoloration producing an equally objectionable appearance;

(d) Not well dried; and,

(e) Not clean.

##### §51.2293 Serious damage.

"Serious damage" means any defect, other than color, which seriously affects the appearance, or the edible or shipping quality of the individual portion of kernel or of the lot as a whole. Any one of the following defects or any combination of defects the seriousness of which exceeds the maximum allowed for any one defect shall be considered as serious damage:

(a) Shriveling when more than one-fourth of the kernel is severely shriveled, or a greater area is affected by lesser degrees of shriveling producing an equally objectionable appearance;

(b) Mold when plainly visible on more than one-eighth of the surface of the kernel in the aggregate; and,

(c) Discoloration of the meat when more than one-fourth the volume of the portion of kernel is severely discolored, or a greater volume is affected by lesser degrees of discoloration producing an equally objectionable appearance.

##### §51.2294 Very serious damage.

"Very serious damage" means any defect, other than color, which very seriously affects the appearance, or the edible or shipping quality of the individual portion of kernel or of the lot as a whole. Any one of the following defects or any combination of defects the seriousness of which exceeds the maximum allowed for any one defect shall be considered as very serious damage:

(a) Shriveling when more than 50 percent of the portion of kernel is severely shriveled;

(b) Mold when plainly visible on more than one-fourth of the surface of the portion of kernel in the aggregate;

(c) Discoloration of the meat when more than one-half the volume of the portion of kernel is severely discolored;

(d) Insect injury;

(e) Rancidity or decay; and,

(f) Shell, or any foreign material.

##### §51.2295 Half kernel.

"Half kernel" means the separated half of a kernel with not more than one-eighth broken off.

##### §51.2296 Three-fourths half kernel.

"Three-fourths half kernel" means a portion of a half of a kernel which has more than one-eighth but not more than one-fourth broken off.

## Appendix A.4



United States  
Department of  
Agriculture

Agricultural  
Marketing  
Service

Fruit and  
Vegetable  
Division

Fresh  
Products  
Branch

# **United States Standards for Grades of Walnuts (*Juglans regia*) in the Shell**

**Effective November 15, 1976**  
*(Reprinted - January 1997)*



## United States Standards for Grades of Walnuts (*Juglans Regia*) in the Shell<sup>1</sup>

### General

- §1.2945 Application.
- §1.2946 Color chart.
- §1.2947 Method of inspection.

### Grades

- §1.2948 U.S. No. 1.
- §1.2949 U.S. No. 2.
- §1.2950 U.S. No. 3.

### Unclassified

- §1.2951 Unclassified.

### Size Specifications

- §1.2952 Size specifications.

### Variety or Type Specifications

- §1.2953 Variety or type specifications.

### Tolerances for Grade Defects

- §1.2954 Tolerances for grade defects.

### Application of Tolerances

- §1.2955 Application of tolerances.

### Definitions

- §1.2956 Practically clean.
- §1.2957 Bright.
- §1.2958 Splits.
- §1.2959 Injury by discoloration.
- §1.2960 Damage.
- §1.2961 Well dried.
- §1.2962 Decay.
- §1.2963 Dark discoloration.
- §1.2964 Rancidity.
- §1.2965 Fairly clean.
- §1.2966 Serious damage.

### General

#### §1.2945 Application.

The standards contained in this subpart apply only to walnuts commonly known as English or Persian walnuts (*Juglans regia*). They do not apply to the walnuts commonly known as black

<sup>1</sup>Packing of the product in conformity with the requirements of these standards shall not excuse failure to comply with the provisions of the Federal Food, Drug, and Cosmetic Act or with applicable State laws and regulations.

#### §1.2276 Color chart.

The color chart (USDA Walnut Color Chart) to which reference is made in §§1.2281 and 51.2282 illustrates the four shades of walnut skin color listed as color classifications.

(a) **Availability of color chart.** The USDA Walnut Color Chart cited in this subpart has been filed with the original document and is available for inspection in the Office of the Federal Register. The color chart is also available for inspection in the Fruit and Vegetable Division, AMS, U.S. Department of Agriculture, South Building, Washington, D.C. 20250, in any field office of the Fresh Fruit and Vegetable Inspection Service of the Fruit and Vegetable Division, or upon request of any authorized inspector of such Service. Copies of the color chart may be purchased from Munsell Color Co., Inc., 2441 North Calvert Street, Baltimore, Md. 21218.

### Grades

#### §1.2277 U.S. No. 1.

"U.S. No. 1" consists of portions of walnut kernels which are well dried, clean, free from shell, foreign material, insect injury, decay, rancidity, and free from damage caused by shriveling, mold, discoloration of the meat or other means. (See §1.2280.)

(a) Color shall be specified in connection with this grade in terms of one of the color classifications. (See §§1.2276, 51.2281 and 51.2282.)

(b) Size shall be specified in connection with this grade in terms of one of the size classifications. (See §§1.2284 and 51.2285.)

#### §1.2278 U.S. Commercial.

"U.S. Commercial" consists of portions of walnut kernels which meet the requirements of U.S. No. 1 grade, except for increased tolerances. (See §1.2280.)

(a) Color of walnuts in this grade shall be not darker than "amber" classification, and color need not be specified. However, color may be specified in connection with the grade in terms of one of the color classifications. (See §§1.2276, 51.2281 and 51.2282.)

(b) Size shall be specified in connection with this grade in terms of one of the size classifications. (See §§1.2284 and 51.2285.)

### Unclassified

#### §1.2279 Unclassified.

"Unclassified" consists of portions of walnut kernels which have not been classified in accordance with either of the foregoing grades. The term "unclassified" is not a grade within the meaning of these standards, but is provided as a designation to show that no grade has been applied to the lot.

### Tolerances for Grade Defects

#### §1.2280 Tolerances for grade defects.

(a) All percentages shall be calculated on the basis of weight.

(b) In order to allow for variations, other than for color and size, incident to proper grading and handling, tolerances shall be permitted for the respective grades as indicated in Table I:

#### §51.2950 U.S. No. 3.

"U.S. No. 3" consists of walnuts in shells which are dry, fairly clean, free from splits, and free from damage caused by broken shells, and free from serious damage caused by discoloration, perforated shells, adhering hulls or other means. The kernels are well dried, free from decay, dark discoloration, rancidity, and free from damage caused by mold, shriveling, insects or other means. (See §51.2954.)

(a) There is no requirement in this grade for the percentage of walnuts having kernels which are "light amber" or "light". However, the percentage, by count, of nuts with kernels not darker than "light amber" (see color chart) which are free from grade defects and/or the percentage with kernels not darker than "light" (see color chart) which are free from grade defects, may be specified in accordance with the facts. (See §51.2954.)

(b) Size shall be specified in connection with the grade. (See §51.2952.)

#### Unclassified

##### §51.2951 Unclassified.

"Unclassified" consists of walnuts in the shell which have not been classified in accordance with any of the foregoing grades. The term "unclassified" is not a grade within the meaning of these standards but is provided as a designation to show that no grade has been applied to the lot.

#### Size Specifications

##### §51.2952 Size specifications.

Size shall be specified in accordance with the facts in terms of one of the following classifications:

(a) **Mammoth size.** Mammoth size means walnuts of which not over 12 percent, by count, pass through a round opening 96/64 inches in diameter;

(b) **Jumbo size.** Jumbo size means walnuts of which not over 12 percent, by count, pass through a round opening 80/64 inches in diameter;

(c) **Large size.** Large size means walnuts of which not over 12 percent, by count, pass through a round opening 77/64 inches in diameter; except that for walnuts of the Eureka variety and type, such limiting dimension as to diameter shall be 76/64 inches;

(d) **Medium size.** Medium size means walnuts of which at least 88 percent, by count, pass through a round opening 77/64 inches in diameter, and of which not over 12 percent, by count, pass through a round opening 73/64 inches in diameter;

(e) **Standard size.** Standard size means walnuts of which not over 12 percent, by count, pass through a round opening 73/64 inches in diameter;

(f) **Baby size.** Baby size means walnuts of which at least 88 percent, by count, pass through a round opening 74/64 inches in diameter, and of which not over 10 percent, by count, pass through a round opening 60/64 inch in diameter; and,

(g) **Minimum diameter, or minimum and maximum diameter.** In lieu of one of the foregoing classifications, size of walnuts may be specified in terms of minimum diameter, or minimum and maximum diameter. **Provided,** That not more than 12 percent, by count, pass through a round hole of the specified minimum diameter, and at least 88 percent, by count, pass through a round hole of any specified maximum diameter.

#### Variety or Type Specifications

##### §51.2953 Variety or type specifications.

The variety or type of any lot, of walnuts in the shell may be specified in accordance with the facts

as follows:

(a) If the lot is of one named variety, that variety name may be specified, **Provided,** That not over 10 percent, by count, of the walnuts in the lot are of another variety or type than that specified; and,

(b) If the lot is a mixture of two or more distinct varieties or types it may be specified as "Mixed Varieties".

#### Tolerances for Grade Defects

##### §51.2954 Tolerances for Grade Defects.

In order to allow for variations incident to proper grading and handling, the following tolerances shall be permitted for nuts which fail to meet the requirements of the respective grades as indicated. Terms in quotation marks refer to color classifications illustrated on the color chart.

Tolerances for Grade Defects			
Grade	External (shell) Defects	Internal (kernel) defects	Color of Kernel
U.S. No. 1...	10 pct. by count for splits. 5 pct. by count, for other shell defects, including not more than 1 pct seriously damaged.	10 pct total by count, including not more than 6 pct which are damaged by mold or insects or seriously damaged by other means, of which not more than 5/8 or 5 pct may be damaged by insects, but no part of any tolerance shall be allowed for walnuts containing live insects.	No tolerance to reduce the required 70 pct of "light amber" kernels or the required 40 pct of "light" kernels or any larger percentage of "light amber" or "light" kernels specified.
U.S. No. 2...	10 pct. by count, for splits. 10 pct. by count, for other shell defects, including not more than 5 pct serious damage by adhering hulls.	15 pct total, by count, including not more than 8 pct which are damaged by mold or insects or seriously damaged by other means, of which not more than 5/8 or 5 pct may be damaged by insects, but no part of any tolerance shall be allowed for walnuts containing live insects.	No tolerance to reduce the required 60 pct or any specified larger percentage of "light amber" kernels, or any specified percentage of "light" kernels.
U.S. No. 3...	Same as above tolerance for U.S. No. 2.	Same as above tolerance for U.S. No. 2.	No tolerance to reduce any percentage of "light amber" or "light" kernel specified.

#### Application of Tolerances

##### §51.2955 Application of tolerances.

The tolerances provided in these standards are on a lot basis, and they shall be applied to a composite sample representative of the lot. However, any identifiable container or group of containers in which the walnuts are obviously of a quality materially different from that in the majority of the containers shall be considered as a separate lot, and shall be sampled separately.

#### Definitions

##### §51.2956 Practically clean.

"Practically clean" means that, from the viewpoint of general appearance, the walnuts are

practically free from adhering dirt or other foreign matter, and that individual walnuts are not damaged by such means. A slightly chalky deposit on the shell is characteristic of many bleached nuts and shall not be considered as dirt or foreign matter.

**§51.2957 Bright.**

"Bright" means a fairly light, attractive appearance. A slight chalky deposit on the shell shall not be considered as affecting brightness.

**§51.2958 Splits.**

"Splits" means walnuts with the seam opened completely around the nut so that the two halves of the shell are held together only by the kernel.

**§51.2959 Injury by discoloration.**

"Injury by discoloration" means that the color of the affected portion of the shell objectionably contrasts with the color of the rest of the shell of the individual nut.

**§51.2960 Damage.**

"Damage" means any specific defect mentioned in this section; or any equally objectionable variation of any one of these defects, any other defect, or any combination of defects which materially detracts from the appearance or the edible or shipping quality of the individual walnut or the lot as a whole. The following specific defects shall be considered as damage:

- (a) Broken shells when the area from which a portion of the shell is missing is greater than the area of a circle one-fourth inch in diameter; or when the two halves of the shell have become completely broken apart and separated from each other;
- (b) Perforated shells when the area affected aggregates more than that of a circle one-fourth inch in diameter. The term "perforated shells" means imperfectly developed areas on the shell resembling abrasions and usually including small holes penetrating the shell wall;
- (c) Adhering hulls when affecting more than 5 percent of the shell surface;
- (d) Discoloration (or stain) which covers, in the aggregate, one-fifth or more of the surface of the shell of an individual nut, and which is brown, reddish brown, gray, or other color in pronounced contrast with the color of the rest of the shell or the majority of shells in the lot, or darker discoloration covering a smaller area if the appearance is equally objectionable;
- (e) Mold when attached to the kernel and conspicuous; or when inconspicuous white or gray mold affects an aggregate area larger than one square centimeter or one-eighth of the entire surface of the kernel, whichever is the lesser area;
- (f) Shriveling when more than 5 percent of the surface of the kernel, including both halves, is severely shriveled, or a greater area is affected by lesser degrees of shriveling producing an equally objectionable appearance. Kernels which are thin in cross section but which are otherwise normally developed shall not be considered as damaged; and,
- (g) Insects when an insect or insect fragment, web or frass is present inside the shell, or the kernel shows distinct evidence of insect feeding.

**§51.2961 Well dried.**

"Well dried" means that the kernel is firm and crisp, not pliable or leathery.

**§51.2962 Decay.**

"Decay" means that any portion of the kernel is decomposed.

**§51.2963 Dark discoloration.**

"Dark discoloration" means that the color of the skin of the kernel is darker than "amber". (See

color chart.)

**§51.2964 Rancidity.**

"Rancidity" means the stage of deterioration in which the kernel has developed a rancid flavor. Rancidity should not be confused with a slightly astringent flavor of the pellicle (skin) or with staleness, the stage at which the flavor is flat but not distasteful.

**§51.2965 Fairly clean.**

"Fairly clean" means that, from the viewpoint of general appearance, the lot is not seriously damaged by adhering dirt or other foreign matter, and that individual walnuts are not coated or caked with dirt or foreign matter. Both the amount of surface affected and the color of the dirt shall be taken into consideration.

**§51.2966 Serious damage.**

"Serious damage" means any specific defect mentioned in this section; or an equally objectionable variation of any one of these defects, any other defect, or any combination of defects which seriously detracts from the appearance or the edible or shipping quality of the walnut. The following specific defects shall be considered as serious damage:

- (a) Discoloration (or stain) which covers, in the aggregate, one-third or more of the surface of the shell of an individual nut and which is brown, reddish brown, gray, or other color in pronounced contrast with the color of the rest of the shell or the majority of shells in the lot, or darker discoloration covering a smaller area if the appearance is equally objectionable;
- (b) Perforated shells when the area affected aggregates more than that of a circle three-eighths of an inch in diameter. The term "perforated shells" means imperfectly developed areas on the shell resembling abrasions and usually including small holes penetrating the shell wall;
- (c) Adhering hulls when affecting more than one-eighth of the shell surface in the aggregate;
- (d) Shriveling when both halves of the kernel are affected by severe shriveling over an area totaling more than one-eighth of the surface; or when both halves are affected over a greater area by lesser degrees of shriveling producing an equally objectionable appearance. When one of the halves of the kernel shows no shriveling, the kernel shall not be considered seriously damaged unless the other half shows shriveling to the extent that over 50 percent of its surface is severely shriveled, or a greater area is affected by lesser degrees of shriveling producing an equally objectionable appearance. Kernels which are thin in cross section, but which are otherwise normally developed shall not be considered as damaged;
- (e) Rancidity or decay; and,
- (f) Uncured kernels which are wet, rubbery and "green".

## Appendix A.5

### Walnut Assessment Guide 2008

How to assess each variety – using 20 walnuts for each batch

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<b>1</b>	<b>Shell attractiveness</b> This is an overall visual assessment on the appearance of the in-shell walnut. Take into account the smoothness, colour, shape and size.	20
<b>2</b>	<b>Shell seal and strength</b> Drop each walnut from shoulder height onto concrete, and then squeeze each walnut to test whether the seal opens. For every walnut that is firmly in tact score 1 point.	20
<b>3</b>	<b>Crackout %</b> Anything 50% or over gets 20 points. Take 2 points off for every 1% down on 50%.	20
<b>4</b>	<b>Ease of extraction</b> Does the kernel come easily out of the shell?	10
<b>5</b>	<b>Kernel shape</b> Does the kernel come easily into halves? Is there uniformity and consistency in the half shape?	10
<b>6</b>	<b>Kernel flavour</b> Pleasant → bland → awful	10
<b>7</b>	<b>Kernel colour</b> Light tan or blond → dark	10
		<hr/>
		100

## Appendix A.6

### Sensory Evaluation Questionnaire

Sex    Male    ☐    Female    ☐

Age range    <19    ☐    19-25    ☐    26-30    ☐    31-50    ☐    >50    ☐

Smoker?    Yes    ☐    No    ☐

How do you drink your coffee?    Strong    ☐    Medium    ☐    Weak    ☐    Not applicable    ☐

How do you drink your tea?    Strong    ☐    Medium    ☐    Weak    ☐    Not applicable    ☐

How much spicy food do you eat?    A lot    ☐    Medium    ☐    Small amount    ☐    Not applicable    ☐

Please evaluate the samples of walnuts, fill in the code number your evaluation for each sample.

#### TASTE

Sample Number	Flavoursome - very pleasant	Flavoursome	Mild taste	Tasteless - bland	Awful

PTO.....

AFTER TASTE

Sample Number	Flavoursome -very pleasant	Flavoursome	Mild taste	Tasteless - bland	Awful

Thank you very much